A New Synthesis of Threonine

By Mikio SATO, Kenji OKAWA and Shiro AKABORI

(Received July 6, 1957)

Despite a little information available concerning the conversion of glycyl residue to threonyl or seryl residue[1-3], there is as yet no satisfactory method for the synthesis of threonine from glycine or glycyl peptides.

In this paper the authors present a new method for the synthesis of threonine from copper glycinate and acetaldehyde. The possibility for such a method has been suggested from the considerations that the methylene group in copper glycinate may be more active than that in free glycine and that the amino group in the copper salt may be protected from unfavorable side reactions. In the established method, the copper salt of glycine is subjected to the reaction with acetaldehyde in a weakly alkaline aqueous solution at 50–70° for 1–2 hours. After removal of copper and other inorganic salts, a mixture of threonine and allothreonine is obtained in a high yield. It seems likely that the reaction proceeds as shown in Fig. 1. The initial reaction of copper glycinate (I) with acetaldehyde appears to give rise to a coordination state (II) of copper threoninate, which is then transformed into a second coordination state (III). The overall reaction equilibrium is shifted in favor of the formation of threonine, since compound III seems to be more stable than compound II especially in alkaline media. These inferences have been drawn from the following observations: (1) while copper threoninate exhibits low pH values in aqueous solution (below 5), copper glycinate is neutral (pH 7.4), (2) the former is adsorbed on an anion exchange resin of OH-form, while the latter is not, and (3) the light absorption of the former is shifted considerably towards shorter wave-lengths as compared with that of the latter (see

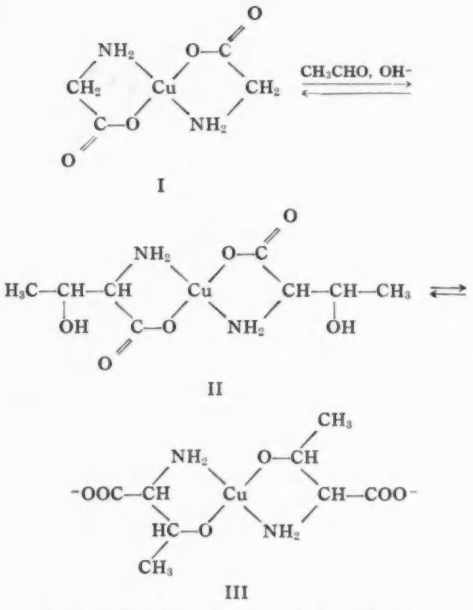

Fig. 1. Mechanism of the threonine synthesis from copper-glycinate and acetaldehyde.

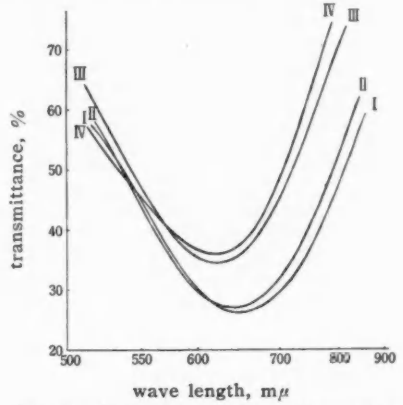

Fig. 2. Absorption spectra of various copper salts (10 μM/ml. of 8% Na_2CO_3 solution). (I), copper glycinate; (II), (I) + CH_3CHO at the beginning of the reaction; (III), (I) + CH_3CHO after the reaction; (IV) copper threoninate.

1) E. Snell, D. Metzler and J. Longenecker, *J. Am. Chem. Soc.*, **75**, 2786 (1953); ibid., **76**, 639 (1954).
2) Th. Wieland and K. Dose, *Angew. Chem.*, **66**, 781 (1954).
3) S. Akabori, K. Okawa and M. Sato, This Bulletin, **29**, 606 (1956).

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Fig. 2). Copper threoninate is in fact bluish purple in color and resembles "biuret" or complexes of amino-alcohols in this respect.

It is expected from the structural formula of compound III that the threo-form of the copper salt may be stereochemically more stable than the allo-form. In fact it has been shown that the former is always produced predominantly over the latter (the ratio of threo/allo being more than 1.6), although the ratio may vary according to the conditions of the reaction. The results presented in this paper may suggest a possible mechanism of the biosynthesis of threonine*. Further experiments are now in progress to find out more favorable conditions for the selective preparation of the threo-form.

Experimental

Copper glycinate $(NH_2CH_2CO_2)_2Cu \cdot H_2O$.—This was prepared by the two following methods. (a) An aqueous solution of glycine was heated with basic cupric carbonate, the excess of the latter was filtered off from the hot solution, the filtrate concentrated in vacuo and cooled. Crystals of copper glycinate thus formed were collected, washed and dried. (b) Glycine (2 moles) was converted into sodium glycinate in aqueous solution and a saturated solution of cupric sulfate (1 mole) was added with stirring. Crystals of copper glycinate deposited were filtered off, washed three times with water and dried.

Copper threoninate.—This was prepared according to the method (a) described for copper glycinate.

Threonine.—A mixture of 115 g. of well-ground copper glycinate, 30 g. of anhydrous sodium carbonate, 370 ml. of water and 160 ml. of freshly distilled acetaldehyde was stirred under reflux on a water bath at 50° for 1 hour. After cooling, excess of acetaldehyde was removed by distillation. One liter of water and 700 ml. of concentrated aqueous ammonia (ca. 15 N) were then added and the solution was filtered. The filtrate was passed through a column of Dowex 50X 12 (H-form, 200-400 mesh, 10 cm×20 cm) which had previously been percolated with 5 N aqueous ammonia. The effluent was concentrated in vacuo and treated with charcoal. The solution was again concentrated in vacuo until the crystallization of threonine began, and then about four volumes of methanol were added. After being kept in a refrigerator for 24 hours, the crystals consisting of both threonine and allo-threonine were collected, washed three times with methanol, and dried in vacuo. The yield was 70 g. From the mother liquor, 6 g of almost the same product was obtained. The total yield was 76 g., or 64 %.

The crystals thus obtained gave, on paper chromatograms, only two spots corresponding to threonine and allo-threonine, except for an insignificant spot due to glycine. Chromatographic analysis of the dinitrophenylated crystals using a column of Amberlite-IRC 50[4] showed that the ratio of threonine to allo-threonine was 1.8.

Anal. Found: C, 40.34; H, 7.14; N, 11.52. Calcd. for $C_4H_9O_3N$: C, 40.34; H, 7.56; N, 11.76%.

In another experiment, a mixture of 115 g. of copper glycinate, 29 g. of potassium carbonate, 230 ml. of water, and 116 ml. of freshly distilled acetaldehyde, was brought to react by heating at 70° for 2 hours. After acidification with hydrochloric acid, the reaction mixture was treated with hydrogen sulfide and filtered. The filtrate was treated with 400 g. of wet Dowex 50 (H-form), and after being freed from water the substanse was dissolved in absolute alcohol. After removal of the precipitated inorganic salts, the free amino acids were precipitated by the addition of aniline. By repeated recrystallization, 27 g. (22.7 %) of crystals (threo/allo=2.4) was obtained. Further 5.5 g. (4.6 %) of crystals (threo/allo=9) was recovered from the mother liquor. The total yield was 32.5 g. (27.3 %).

Summary

A new method is described for the synthesis of threonine from acetaldehyde and copper glycinate. This method gives a mixture of allo-threonine and threonine in a high yield (64 %).

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* After we completed this manuscript, we have become aware of a recent report by J. B. Gilbert (*J. Am. Chem. Soc.*, **79**, 2242 (1957)) which shows that a mixture of allo-threonine and threonine can be formed from acetaldehyde and glycine in the presence of an enzyme preparation from rat liver.

4) T. Seki, to be published.

A New Balancing Drop Method for the Determination of the Density of Heavy Water

By Yoshihide NAITO

(Received June 28, 1957)

The usual falling drop method[1] for the determination of the density or the deuterium-concentration of heavy water is based upon Stokes' law; the falling velocity of a drop of sample water through a suitable organic liquid is compared with that of standard water. Consequently in this method the size of the drop must be kept strictly constant and the falling velocity must be measured very accurately. Furthermore, the temperature of the working room must be kept as constant as possible in order to secure the equilibration of temperature between the falling drop and the surrounding organic liquid medium. To avoid such troubles we have tried a new method, in which the drop of the sample water is brought to balance in the liquid medium by changing the temperature of the latter as in the case of the float method[2]. Then the density of the sample can easily be known from the temperature dependence of the density of the medium liquid.

The main part of the apparatus is shown in the appended diagram. It consists of an ordinary glass tube about 30 cm. long and 13 mm. wide, the lower end of which is hermetically closed. The tube is provided with a glass stopper S at the upper end and the lower part of the tube is surrounded with a glass jacket J, through which cooling water can be circulated. A suitable organic liquid or a liquid mixture, the density of which is almost equal to that of the sample water, is placed in the tube up to marking M and the whole is immersed in the water of the thermostat down to the level W, while cooling water is circulated through the water jacket J. But as the temperature of the cooling water is kept somewhat lower than that of the thermostat, the density of the liquid column in the tube between H and K is always greater than that of the same

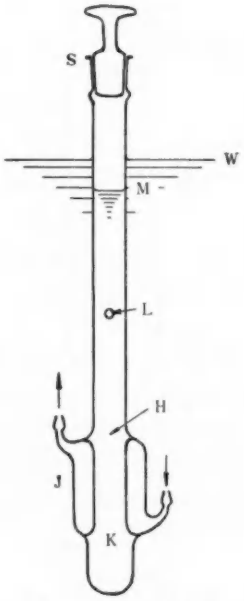

Fig. 1. Apparatus

liquid between M and H. In other words there is a discontinuity in the density of the liquid column at the point H. It is not difficult to choose the temperature of the thermostat in such a way that the density of the liquid column above the point H is less, while that below the point H is greater than that of the sample water, so that the drop of sample water, which has been dropped into the tube, remains at the point H in the liquid column. In this way the drop of water is prevented from falling and adhering to the bottom of the tube.

Now the temperature of the thermostat is carefully lowered and in this way the density of the liquid column in the tube between M and H is gradually increased. Then the drop of the water, which has been allowed to remain at first at the point H, gradually floats up and finally under some adjustment of the temperature it

1) H. G. Barbour and W. F. Hamilton, *Am. J. Physiol.*, **69**, 654 (1924).

2) Such a control of the temperature can be made, because the thermal expansion coefficient of organic liquids is generally greater than that of water.

stops at a certain point (L) in the liquid column between M and H. When this balancing temperature is read off from Beckmann's thermometer immersed in the thermostat, the density of the sample water can easily be known from the temperature dependence of the organic liquid in the tube.

TABLE
EXAMPLES OF THE MEASUREMENTS
Deuterium-concentration atom %

By the pycnometric method	By the present method
0.015*	0.02±0.01**
0.25	0.25±0.01
0.49	0.48±0.01
0.70	0.70±0.02
0.94	0.96±0.01
1.17	1.16±0.01
1.40	1.40±0.01
1.67	1.66±0.01
1.87	1.89±0.02
2.31	2.31±0.00**

* Natural water.
** These samples were used as standards.

Some examples of the determinations are given in the appended table, where the deuterium-concentration of various dilute heavy waters is determined by the present method and the results are compared with those obtained by the ordinary pycnometric method. As an organic liquid, a mixture of 100 parts of chlorobenzene and about 60 parts of xylene was used and the balancing temperature lay at about 35°C.

As will be seen from the table, deuterium-concentration of water between 0—3 atom per cent. can easily be measured by the present method with a relative error of 1%. One of the advantages of the present method over the usual falling drop method is that an accurate micropipette for the making of the drop is unnecessary. An ordinary pipette or a syringe is enough for the making of a drop of water of an arbitrary size for the present method. Furthermore, at the end of an experiment, the water in the liquid column can easily be sucked off by means of a pipette after bringing the drop on the surface of the liquid column by slightly lowering the temperature of the thermostat.

The author wishes to express his gratitude to Professor T. Titani for much help and encouragement in this work.

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Heat of Mixing of Mono- and Di-propylene Glycol with Water

By Kazuo AMAYA and Ryoichi FUJISHIRO*

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It will be important to investigate what changes of physical properties of the solutions occur according to the size of the solute molecules. As an attempt we measured the heat of mixing of propylene glycol and di-propylene glycol with water. The di-propylene glycol molecule is thought to be about twice as large as that of propylene glycol; both molecules have the same number of hydroxyl group, but are different in that the former has the ether group.

Measurements of heats of mixing of these solutions, therefore, will give some interesting bits of information about the effect of the molecular size and the interaction power of the ether and the hydroxyl groups with water.

Experimental

The apparatus used was already described in the previous paper[1]. Propylene glycol and di-propylene glycol were distilled with a long column under reduced pressure.

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1) K. Amaya and R. Fujishiro, This Bulletin, **29**, 270 (1956).

Results and Discussion

The heat which was evolved when propylene glycol (or di-propylene glycol) and water were mixed and then when the solution was diluted successively with propylene glycol (or di-propylene glycol) or water, was measured at 25°C. By integrating these heats evolved by mixing and dilution, the heat of mixing at various concentrations was obtained as shown in Tables I and II. For reference, the heat of mixing and dilution of tri-propylene glycol with water was also measured. But tri-propylene glycol was not distilled because of its high boiling point and so the data may not be reliable enough. Using these data, the heat of mixing per mole of solution was calculated and plotted in Fig. 1.

Table I

Heat of mixing of propylene glycol and water at 25°C

W. of P.G. g.	W. of water g.	Mole fraction of PG	Heat evolved cal.
1.670	15.649	0.0247	45.9_7
1.670	10.131	0.0376	43.7_1
1.670	6.082	0.0611	39.5_2
1.670	4.018	0.0896	34.7_2
1.670	2.079	0.1598	24.5_2
1.996	2.055	0.1870	27.5_1
4.027	2.055	0.3170	31.8_7
7.958	2.055	0.4784	34.8_3
15.010	2.055	0.6336	37.4_5

Table II

Heats of mixing of di-propylene glycol and water at 25°C

W. of di-PG g.	W. of water g.	Mole fraction of di-PG	Heat evolved cal.
1.991	16.496	0.0160	52.6_2
1.991	8.277	0.0313	46.8_9
1.991	4.178	0.0602	36.0_0
2.058	2.002	0.1213	28.2_0
4.146	2.002	0.2176	30.6_0
8.073	2.002	0.3513	30.7_1
14.813	2.002	0.4984	30.7_4

It will be found that the di-propylene glycol solution shows different and characteristic behavior compared with the propylene glycol solution. One of these behaviors is the shift of the maximum position of the curve to the lower concentration of di-propylene glycol and the other is the increase in the maximum height in the heat of mixing.

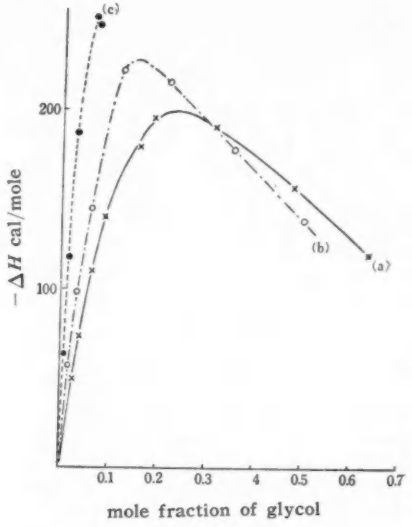

Fig. 1.
(a) Propylene glycol-water
(b) Di-propylene glycol-water
(c) Tri-propylene glycol-water

Usually the heat of mixing per unit volume of the solution of two kinds of normal liquid which have no specially interacting group, may be represented by the following van Laar equation:

$$\Delta H_m/cc = \alpha v(1-v) \qquad (1)$$

where α is the interaction parameter between the solute and the solvent molecules and v is the volume fraction of the solute. According to this equation the position of the maximum of $|\Delta H_m/cc|$ will be at the equi-volume concentration. Assuming that the ratio of molecular volume of the solute to the solvent be n, the heat of mixing per mole of solution will be rewritten as the function of mole fraction x by

$$\Delta H_m/\text{mole} = V_0\alpha x(1-x)/\{1-(n-1)x\}$$

where V_0 is the molar volume of the solvent. Accordingly, the maximum of ΔH_m/mole may be easily calculated to be at $x_{max}=(\sqrt{n}-1)/(n-1)$, which shows that x_{max} decreases as the molecular size of the solute increases.

When one or both liquids have specific groups of interaction, the value of x_{max} will deviate more or less from these normal ones with the degree of interaction, because in such cases the van Laar equation can not be applied owing to the deviation from random mixing.

The propylene glycol-water and the di-propylene glycol-water system which have the effective hydroxyl groups, may be such cases, and the deviation from the normal behavior in both cases is supposed to be considerable.

The ratio n of the molecular volume of propylene glycol or di-propylene glycol to water being 4.1 or 7.2, x_{max} is expected to be 0.33 or 0.27 respectively, which should be compared with the observed value 0.24 or 0.15.

It will not be surprising that both the observed values deviate in similar order from the normal ones and that the observed value of x_{max} for the di-propylene glycol-water system is lower than that for the propylene glycol-water system. It seems strange that the height of the maximum for the di-propylene glycol-water system is higher than that for the propylene glycol-water system, as di-propylene glycol and propylene glycol have the same number of hydroxyl groups which are supposed to show the main interaction with water. But the di-propylene glycol molecule has the oxygen atom making the ether bonding in its center, while the propylene glycol molecule has not. This oxygen atom in the di-propylene glycol molecule may be supposed to play an important role as an acceptor of hydrogen bonding with water, and to make a considerable contribution to the heat of mixing.

This remarkable power of the oxygen atom may be seen in the tri-propylene glycol-water system shown in Fig. 1, in which the former molecule has two such oxygen atoms making the ether bonding.

In order to make a further study of this fact, the heat of mixing per mole of propylene glycol or di-propylene glycol was calculated from the experimental data and plotted against the concentration of propylene glycol or di-propylene glycol in Fig. 2. In the low concentration range, the plots prove to be nearly linear. Extrapolation to the infinite dilution of propylene glycol or di-propylene glycol gives 2.3 kcal. and 4.1 kcal. for the propylene glycol-water solution and the di-propylene glycol solution respectively. This great difference of 1.8 kcal. may be attributed to the existence of the oxygen atom making the ether bonding in the di-propylene glycol molecule.

By considering di-propylene glycol as two unit molecules of propylene glycol, the heat of mixing per unit mole of solution (number of water molecules plus two times number of the di-propylene glycol molecules equal to a mole) was calculated from the experimental data and shown in Fig. 3 with that of the propylene glycol system. It will be seen that the curves for both systems nearly

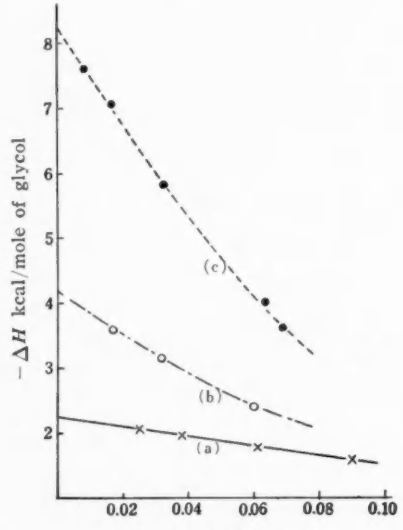

Fig. 2.
(a) Propylene glycol-water
(b) Di-propylene glycol-water
(c) Tri-propylene glycol-water

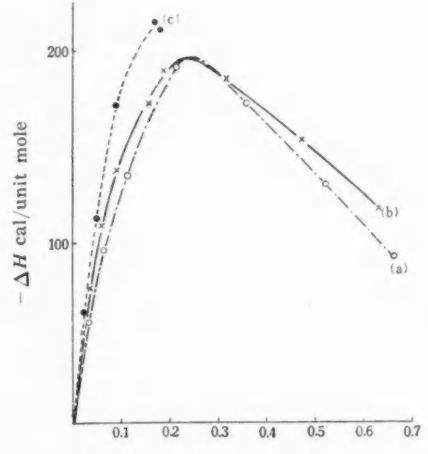

Fig. 3.
(a) Propylene glycol-water
(b) Di-propylene glycol-water
(c) Tri-propylene glycol-water

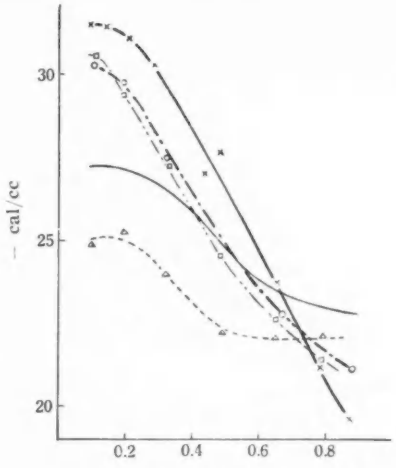

Fig. 4.
—×— Propylenglycol-water
-○- - Di-propylene glycol-water
-●-·· 1.3-Butanediol-water
—— Ethylene glycol-water
- -△- - 1.4-Butandiol-water

coincide in the height and in the position of the maximum. It may be reasonable to suppose that the di-propylene glycol molecule shows the same behavior as that of two units of a propylene glycol molecule and hence the oxygen atom in the ether bonding has the same power of interaction for water as that of two hydroxyl groups.

Finally, we shall compare the interaction parameter α of our two systems with those of the solutions containing such molecules as ethylene glycol[2] and butanediol[3] which have two hydroxyl groups. Using Eq. (1) the values of α for several systems were calculated and illustrated in Fig. 4. The similar behaviors in cases where the values of α are fairly great in magnitude and decrease considerably as the concentration of diol increases, will be found for all systems shown in Fig. 4 and hence may be characteristic of these solutions in which hydroxyl groups play an important role.

Summary

The heat of mixing of propylene glycol and di-propylene glycol with water was measured. The results obtained for these systems were compared with each other and it was found that the oxygen atom in the ether bond of di-propylene glycol plays an important role for the acceptor of hydrogen bonding and makes a great contribution to the heat of mixing.

We would like to express our gratitude to Professor Y. Morino of Tokyo University for his encouragment and discussion.

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2) Schwers, *Rec. trav. chim.*, **28**, 44 (1909).
3) K. Amaya and R. Fujishiro, This Bulletin, **29**, 830 (1956).

The Static Electrode Potential Behavior of Tantalum and its Behavior on Anodic Polarisation

By E. M. Khairy and M. Kamal Hussein

(Received October 10, 1956)

As is well known, tantalum shows remarkable resistivity towards chemical action and behaves frequently as a passive metal. A study of its static electrode potential behavior and of its anodic polarization would be of importance for obtaining insight into its mode of oxidation leading to the observed resistivity. For this purpose, the electrode potential of tantalum was measured in buffer solutions covering the pH range 1—12 and initially free from its ions. The repetitive oscillographic method was also applied to study its anodic behavior in various media.

Experimental

The electrodes used in this investigation were pure tantalum wires each 1 cm. long and 1 mm. in diameter. The static electrode potential measurements were performed at 30° C in the Clark and Lub's-Ringer series of buffers covering the pH range 1—12. Before use the electrodes were polished with different grades of emery paper, thoroughly washed with redistilled water and finally rinsed with the solution to be used. Cells and devices were essentially the same as those used in previous papers[1]. A saturated calomel electrode was used as the reference half cell.

The anodic polarization experiments were carried out at 30° C by the repetitive oscillographic method, using the same circuit as that applied by us in a previous publication[2]. The trip voltage of the thyratron valve was always adjusted at 65 V. The electrolytes used were buffer solutions chosen in the acid, the neutral and the alkaline range, together with buffered potassium fluoride of concentrations ranging from 10^{-2} to 5 N. Polarization curves were also taken in several unbuffered electrolytes including hydrofluoric, hydrochloric, sulfuric and nitric acid and their neutral salts. All these electrolytes were prepared from the purest Analar products.

Results and Discussion

The Electrode Potential Behavior of Tantalum in Solutions Initially Free from its Ions.

—The electrode potential of tantalum in buffer solutions varies with time in such a way that the first steady state values are generally characterised in acid media by considerable drifts towards more negative overnight values. Alternatively, the potentials obtained in neutral and slightly alkaline media show pronounced drifts towards more positive values when the electrodes were left overnight in contact with the electrolytes.

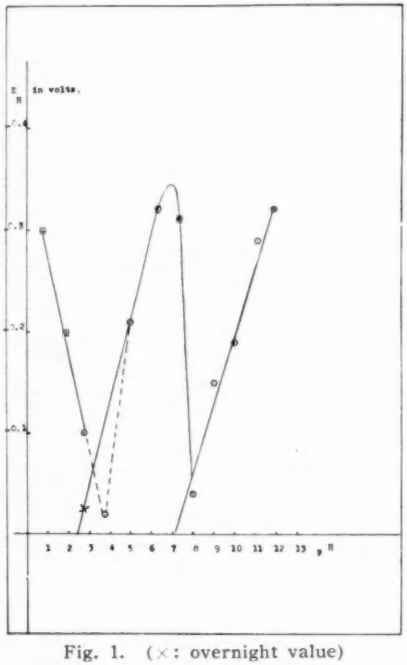

Fig. 1. (×: overnight value)

By plotting the first steady state potentials against pH, a "W"-shaped curve as shown in Fig. 1 is obtained. It consisits of a more or less linear but rather rapid fall of potential within the pH range 1 to 3. The curve then passes through a sharp peak whereupon the potential varies linearly up to pH 7, yielding a coefficient

1) A. Riad Tourky and S. E. S. El Wakkad, *J. Chem. Soc.*, **1948**, 740.
2) W. Machu, E. M. Khairy and M. K. Hussein, *Corrosion*, U. S. A., **11**, 3751 (1955).

of 81 mV per unit pH. Another straight line can be traced along the pH range 8—12, the slope of which amounts to 66 mV per unit pH. By extrapolating the lines running across the pH ranges 3—7 and 8—12, E'_0 values are obtained amounting to +0.192 and +0.473 V, respectively.

In order to interpret the phenomena manifested by the tantalum electrode, the thermodynamic potentials of the systems Ta/Ta_2O_5 and $Ta/Ta_2O_5/TaO_2^+$ have been computed. According to the calculations reported by Latimer (3), the E_0' value of the former system amounts to −0.8114 V. In order to evaluate the thermodynamic potential of the latter system, the following equilibrium reactions have been considered:

$$Ta_2O_5+H_2O \rightleftarrows 2\,TaO_2^+ +2OH^- \quad (1)$$

By applying the relation

$$\log K=-(2\mu^0_{TaO_2^+}+2\mu^0_{OH^-}-\mu^0_{Ta_2O_5}-\mu^0_{H_2O})/1{,}363 \quad (2)$$

and using Latimer values for μ^0 potentials of Ta_2O_5, OH^- and H_2O which amount to −470.6, −37.596 and −56.690 kcal., respectively it follows that:

$$\mu^0_{TaO_2^+}=-226.050-0.682\log K \quad (3)$$

Using this value, the E_0' value of the reaction:

$$Ta+2H_2O=TaO_2^+ +4H^+ +5e^- \quad (4)$$

amounts to:

$$E_0'=-0.9763-0.0059\log K \quad (5)$$

Assuming that the electrode potential of tantalum is determined by the activity of the tantalyl ion in solution, it follows that:

$$E_H=E_{0(Ta/TaO_2^+)}+\frac{RT}{F}\log a_{(TaO_2^+)} \quad (6)$$

Substituting for $a_{(TaO_2^+)}$ from the relation:

$$a_{(TaO_2^+)}=\frac{K^{1/2}}{a_{(OH^-)}}$$

where K denotes the equilibrium constant of reaction (1), equation (6) transforms into:

$$E_H=-0.9763-0.0059\log K+\frac{RT}{F}\log\frac{K^{1/2}}{a_{OH^-}}$$

$$=-0.9763+0.0236\log K-0.059\log\frac{K_w}{a_{H^+}}$$

$$=-0.1503+0.0236\log K-\frac{RT}{F}pH \quad (7)$$

or

$$E_H=E_0'-\frac{RT}{F}pH \quad (8)$$

where

$$E_0'=-0.1503+0.0236\log K \quad (9)$$

The results obtained with the tantalum electrode in buffer solutions initially free from its ions may be explained in view of its decided tendency to acquire the passive state (4). This state is most probably attained when the electrode is covered with an inhibitive film of the pentoxide overlayered with adsorbed oxygen doublets (5). The presence of such a layer together with the passive nature of the surface oxide, accounts for the observed surplus in potential. In the light of the above discussion, one may interpret the E_H/pH plot as follows: from pH 1 to 3, the electrode which has been rendered passive by exposure to atmospheric oxygen is subjected to the dissolving action of the electrolyte. This type of attack which takes place through the pores of the pre-immersion film, proceeds with subsequent depolarisation of some of the oxygen doublets, an effect which becomes less pronounced with increase in pH. This makes up for the shift of potential towards more positive values within that range. The electrodic behavior may be conceived under such conditions as that of a metal/metal pentoxide electrode superimposed by varying contributions of overvoltage brought about by the adsorbed oxygen doublets. At pH 3 upwards, the attack is materially diminished and the passive state seems to predominate, the electrode behaving thus as a $Ta/Ta_2O_5/O_2^-$ at a more or less constant overvoltage effect. On comparing the E_0' value obtained within the pH range 3—7 (0.192 V) with the thermodynamic potential of the Ta/Ta_2O_5 couple (−0.8114 V), it appears that the overvoltage contributed by the adsorbed gas layer and/or the polarisation brought about the passive nature of the surface film, amounts to as high a value as one volt.

Owing to the complexity of the quinquivalent tantalum ion, it is legitimate to assume that the metal as well as the oxide dissolve in alkaline media in the form of tantalyl ions (TaO_2^+), according to the reaction:

$$Ta+2H_2O=TaO_2^+ +4H^+ +5e^- \quad (i)$$

3) W. M. Latimer, "Oxidation Potentials", 265 (1953).
4) R. Audubert and M. L. Roulleau, *J. Chim. Phys.*, **37**, 101 (1940).
5) G. Gesterheld, *Z. Electrochem.*, **11**, 47, 503 (1905).

and the equilibrium reaction:

$$Ta_2O_5 + H_2O \rightleftarrows 2\,TaO_2^+ + 2\,OH^- \qquad (ii)$$

Supposing that the behavior along the pH range 8—12 is given by the system $Ta/Ta_2O_5/TaO_2^+$ and assuming that the overvoltage contributed by the adsorbed doublets acquires but little variation with pH, the equilibrium constant K of the reaction (ii) may be evaluated. Thus by substituting for E_0' in equation (9) by the value computed from the slope of the respective straight line from which one volt (the difference between the experimental and the theoretical potential) is subtracted, it follows that:

$$0.473 - 1.0 = -0.150 + 0.024 \log K.$$

Therefore,

$$\log K = 15.7,$$

from which $K = 5 \times 10^{-16}$.

The Anodic Behavior Investigated by the Repetitive Oscillographic Method. —The study of the anodic behavior of tantalum by the aid of the cathode ray oscillograph reveals generally the presence of three domains. The first domain comprises two main stages, one of which occurs at a more or less constant negative potential corresponding to the desorption of hydrogen supposedly formed through the cathodic action of the repetitive current. The second stage in this domain consists of a gradual potential rise varying regularly with the applied quantity of electricity, a stage to be described to the building up of the double layer potential. The second domain in these curves includes the stages corresponding to the different states of oxidation of the metal, whereas the third domain covers the regions of attainment of passivity and gas evolution. The results of these polarisation experiments may be outlined as follows:

a) *Experiments in Buffer Solutions*:—The anodic behavior of tantalum was investigated in different buffer solutions within the pH range 1—14. The oscillograms obtained were essentially of the same general form, showing that the electrode maintains its passive nature all over the pH range. Figs. 2—5 contain representative curves taken, successively, in solutions of pH 1, 7, 12 and 14 at the polarising current densities 8.2, 6.2, 13.8 and 7 mA/cm². The appropriate amounts of electricity were supplied by means of a 0.2, 0.4, 1 and 1 μF/cm² condensers, respectively.

On comparing the first domains of these curves, it may be noted that the curve obtained at pH 14, in contradistinction from the other three curves, is characterised by a rather extended stage corresponding to the desorption of hydrogen. This may be explained by assuming that the natural oxide is partially attacked during the initial stages of the anodic process owing to the tendency of tantalum pentoxide to form tantalate in the strong alkaline media. The process takes place with the formation of hydrogen which is sorbed by the metal, together with the

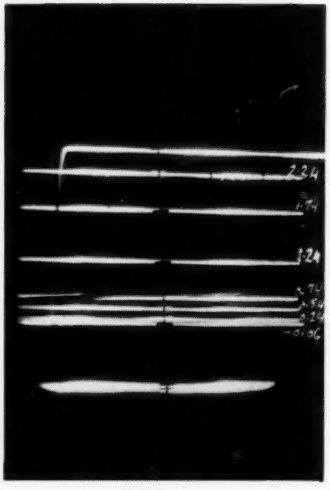

Fig. 2

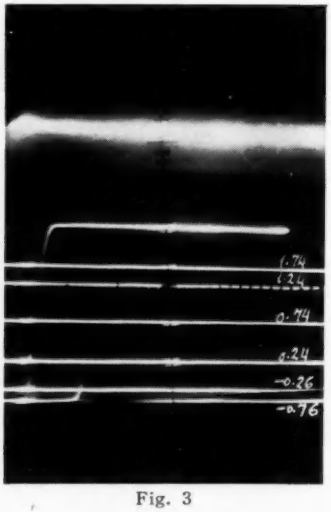

Fig. 3

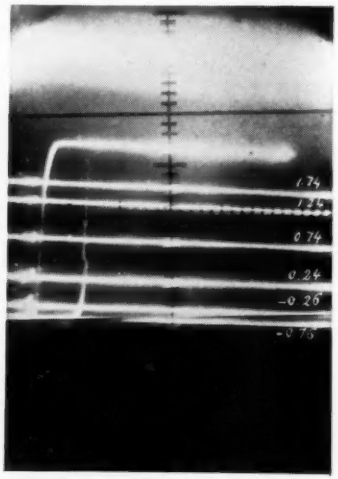

Fig. 4

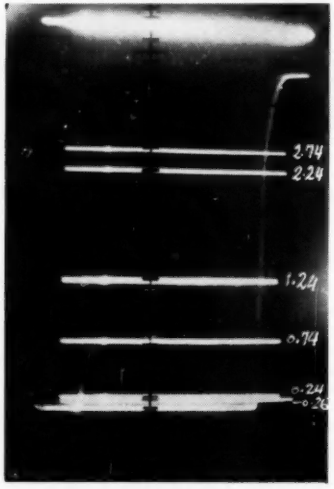

Fig. 5

gas evolved during the cathodic passage of the repetitive current. The removal of the sorbed gas consumes comparatively greater amounts of electricity.

After the process of desorption of hydrogen, the potential jumps abruptly towards appreciably more positive values, attaining a value ranging from 2.5 to 3.5V at which oxygen evolution takes place. This indicates that the electrode surface is passivated by the time the double layer has been fully charged. The capacity of the double layer may be calculated for each curve from the slope of the respective linear potential rise, the horizontal shift of the cathode ray track being proportional to the quantity of electricity and the vertical shift to the acquired potential. The total amount of electricity used up in the overall process is equal to the product of the trip voltage of the thyratron valve (65 V) and the capacity of the applied condenser. The values thus obtained are almost the same averaging around 1 μF/cm^2 of the apparent surface area. The ease with which oxygen is evolved together with the exceedingly low double layer capacities substantiates the passive nature of the electrode surface. This behavior corroborates the results of the static potential measurements regarding the persistence of a naturally present protective oxide film on the electrode surface.

b) *Experiments in Solutions Containing Fluoride Ions*:—Anodic polarization experiments have been carried out in potassium fluoride solutions buffered within the pH range 2—12 with the object of investigating the corroding effect of the fluoride ion when present at lower and higher concentrations (10^{-2}--5 M). The oscillograms thus obtained are essentially of the same shape inasmuch as pronounced passivity is achieved in both dilute and concentrated solutions. A representative curve is shown in Fig. 6. It contains two curves "A" and "B" being taken in 10^{-2} and 5 M potassium fluoride solutions buffered at pH 7, both at a current density of 9.2 mA/cm^2 together with a 0.3 μF condenser. The potential jumps abruptly after the double layer has been fully charged and oxygen is readily evolved at 3.5 V, consuming the

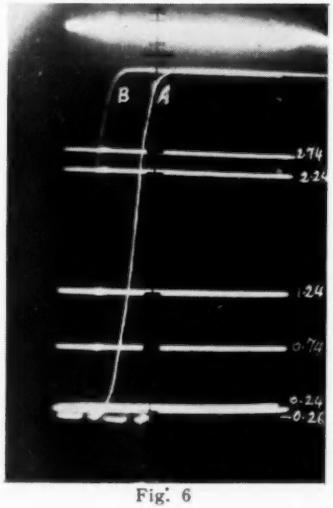

Fig. 6

most part of the applied quantity of electricity. This shows that the fluoride ion introduces no appreciable effect on the attainment of anodic passivity even at the higher concentration. Therefore, it appears legitimate to extend the study to a still higher fluoride ion concentration. For this purpose the electrode was anodically polarized in 10 N hydrofluoric acid, whereupon two oscillograms "C" and "D" were obtained (Fig. 7), taken at 10.5 and 9.4mA/cm^2 together with a 0.3, respectively a 1 μF condenser. Consideration of these curves reveals that when the applied quantity of electricity is relatively small (curve C), the electrode acquires a rather active potential of ~0.2 V after the double layer has been built up, indicating that

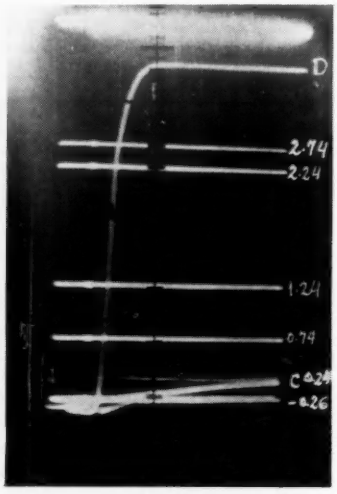

Fig. 7

the corroding effect exerted by the fluoride ion hampers the achievement of passivity. The capacity of the double layer amounts to 30 μF/cm^2 as compared with 1 μF/cm^2 in buffered potassium fluoride solutions, revealing the relative activity of the surface. The electrode behavior may be conceived under such conditions as to be governed by a tantalum/tantalum pentoxide system subject to some overvoltage effect, a picture which conforms to the static electrode potential behavior. Alternatively, prominent passivity is readily achieved on passing higher quantities of electricity. The capacity of the double layer amounts to a value of 3 μF/cm^2 and oxygen is evolved at 3.5 V.

c) *Experiments in Chloride, Sulfate and Nitrate Media*:—The oscillograms shown in Figs. 8 and 9 were taken in 6 N hydrochloric acid and 1 N sodium chloride solutions. The applied quantities of electricity were 9.7 and 6.5 mA/cm^2, using a 0.3 and 1 μF condenser, respectively. These curves show also that the electrode surface acquires the passive state simultaneously as the double layer has been fully charged, oxygen being evolved at ~4 V. The double layer capacity amounts to 1.5 and 1.1 μF/cm^2 for the acid and the neutral solutions, respectively.

The electrode shows a remarkably

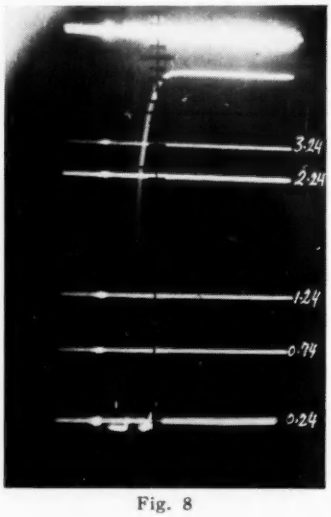

Fig. 8

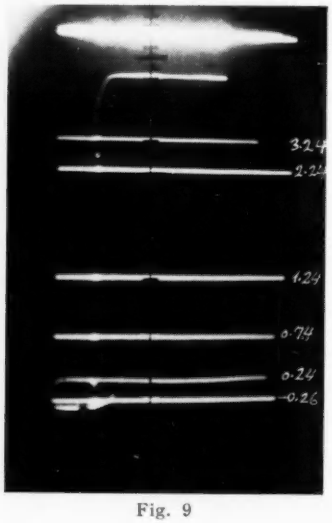

Fig. 9

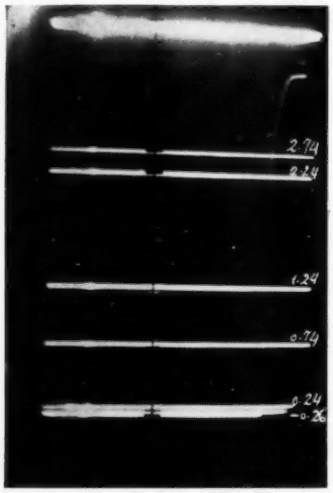

Fig. 10

similar behavior in 1 N sulfuric and nitric acid solutions. A representative curve is shown in Fig. 10 which was taken in 1 N sulfuric acid at 4 mA/cm² together with a 0.5 μF condenser. It is apparent that although passivity is rapidly attained, as was the case in chloride media, yet the first domain in this representative curve manifests a rather extended stage which corresponds to the desorption of hydrogen. This indicates that the activation encountered by the repetitive nature of the current is much more effective in sulfuric and nitric rather than in hydrochloric acid. It has to be inferred, however, that on carrying out experiments in sodium sulfate and sodium nitrate solutions, the curves obtained are essentially the same as those given in the sodium chloride solutions (Fig. 9), owing to the diminished dissolving action of these neutral electrolytes.

Summary

The electrode potential behavior of tantalum is investigated in buffer solutions initially free from the metal ions covering the pH range 1—12. A "W"-shaped E_H/pH plot is obtained and the results reveal the existence of a natural passive pentoxide film overlayered with oxygen doublets. The electrode behaves within the pH range 3—7 as a metal-metal oxide electrode subject to an overvoltage of about one volt. From pH 7 to 12 the behavior is given by the system: Ta/TaO_2^+ yielding an equilibrium constant $K=5.10^{-16}$.

Anodic polarization experiments by the repetitive oscillographic method have been also utilised in buffer solutions and in other different media. The oscillograms are mostly characterised by sharp potential jumps associating the charging of the double layer, showing the decided resistivity of the surface film. The capacity of the double layer scarcely exceeds 1 μF/cm², except in hydrofluoric acid solutions where it attains a maximum value of 30 μF/cm².

The authors wish to express their full gratitude to Professor Dr. A. Riad Tourky, Head of the Chemistry Department for his valuable criticism and cordial facilities in carrying out this work.

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The Photochemical Degradation of 6 Nylon. I*

By Takeshi HASHIMOTO

(Received July 10, 1957)

The photochemical degradation of nylon has been the subject of several recent publications[1-3]. This topic has been under investigation in this laboratory for some time, and the author has found that the photochemical degradation of 6 nylon is accompanied with considerable decrease in molecular weight and increase in water soluble material. The water soluble material of nylon consists of monomers and oligomers. In this paper it is attempted to elucidate the mechanism of the photochemical degradation of 6 nylon from the relationship between molecular weight and the amount of monomer produced.

Various Possible Modes of Linear Polymer Degradation

Grassie and Melville[4] made a consideration of various possible modes of molecular weight change the starting point in their study of the reaction. Before appreciable amounts of monomer appear in a random scission process in large molecules, the number-average molecular weight of the residue is reduced to a small fraction of its initial value. This behavior is represented by AD in Fig. 1. Another suggestion was that the bonds joining the end units to the rest of the chain might be particularly vulnerable and thus exclusively broken. Such a process has been called stepwise depolymerization and would result in decrease in molecular weight, directly proportional to the amount of monomer produced. This behavior is represented by AC in Fig. 1.

A third possible depolymerization mechanism is the exact reverse of polymerization. Initiation would consist of chain scission resulting in the production of radicals. These would then rapidly lose monomer until they had completely disintegrated. If these degrading radicals had a lifetime of the order of those of the growing radicals in polymerizing systems (approximately 10^{-3} —10 sec.), virtually all the monomer units would exist at any instant as free monomer or unchanged polymer. No great change in the molecular weight of the residue would therefore be expected throughout the whole course of the reaction. This mechanism is represented by AB in Fig. 1.

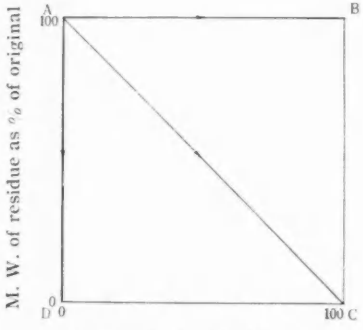

Fig. 1. Theoretical reaction curves for possible depolymerization mechanisms (Grassie and Melville).

Experimental

Scoured and heat-set nylon taffeta of 70D 34f(1) 6 nylon yarn (Amilan) was used in these experiments. Samples of the fabric were irradiated in a Fade-Ometer for various periods of time and then the amount of water-soluble material and the degree of polymerization of the sample were measured.

1) *The measurement of the amount of water soluble material.*—Half a gram, of irradiated nylon taffeta was dried in vacuo over phosphorous pentoxide for 24 hr. and weighed. After 4 hr. extraction with water in a Soxhlet extractor, decrease in the weight of the sample was referred to as the amount of water soluble material.

2) *The measurement of the degree of polymerization.*—Number-average degree of polymerization $\bar{P}_n$ is calculated from intrinsic viscosity

* This paper was read before the 10th Annual Meeting of the Chemical Society of Japan held in Tokyo, April, 1957.

1) B. G. Achhammer et al., *J. Appl. Chem.*, **1**, 301 (1951).
2) R. A. Ford, *Nature*, **176**, 1023 (1955).
3) M. Schwemmer, *Textil-Rundschau*, **11**, 1, 70, 131 (1956).
4) N. Grassie and H. W. Melville, *Proc. Roy. Soc.* **A 199**, 1, 14, 24 (1949).

TABLE I

WATER-SOLUBLE MATERIAL AND NUMBER AVERAGE DEGREE OF POLYMERIZATION OF IRRADIATED NYLON

Exposure time (hr.)		0	10	20	40	60	80	120
Sample weight (mg.)		490	458	487	487	487	484	488
Water-soluble (%)		0.7	0.8	1.1	1.9	2.9	3.6	5.6
Material								
$[\eta]$	Before extraction	1.02	0.70	0.50	0.33	0.17	0.30	0.19
	After extraction		0.73	0.65	0.42	0.31	0.34	0.23
$\bar{P}_n$	Before extraction	131	76	47	26	10	22	12
	After extraction		81	68	36	23	27	15

$[\eta]$ which is measured in *m*-cresol at 25.00°C. Thus $\bar{P}_n = 127.4\,[\eta]^{1.435}$ [5].

Results

All the results are summarized in Table I.

Fig. 2 shows the increase in amount of water-soluble material and the decrease in intrinsic viscosity during irradiation. The increase in amount of water-soluble material is approximately proportional to the exposure time, while the decrease in intrinsic viscosity is rapid at the beginning of exposure. It might be due to the presence of water-soluble material that the intrinsic viscosity after extraction always shows a little higher value than that before extraction.

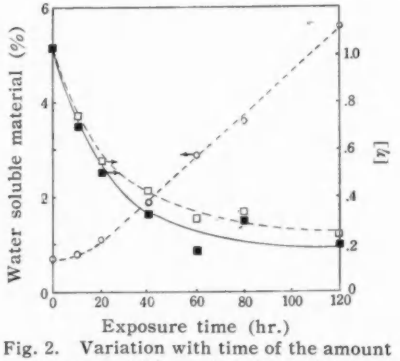

Fig. 2. Variation with time of the amount of water-soluble material and intrinsic viscosity (○ water soluble material, ■ intrinsic viscosity before extraction, □ intrinsic viscosity after extraction).

The relationship between the amount of water soluble material and the number-average degree of polymerization after extraction is shown in Fig. 3. Monomer is not the only water-soluble material produced. From the analogy of Fig. 1, however, it is deduced that the molecular chain of nylon is randomly split off by light.

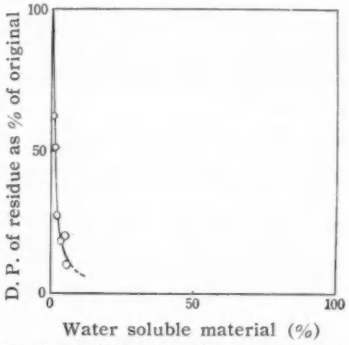

Fig. 3. Relationships between the amount of water-soluble material and the number-average degree of polymerization of residue.

Discussion

A theory regarding the random breaking of polymer chain has been elaborated by a number of workers. The theory makes it possible to obtain mathematical expressions for the various size distributions at definite stages of the degradation process. Here is cited Hoshino and Watanabe's[6] theory from which the relationship between the amount of monomer or oligomer produced and the number-average degree of polymerization is derived in the following way.

Supposing the molecular size distribution at initial stage follows Flory's distribution. That is

$$N = N_p^{x-1}(1-p) \qquad (1)$$

where N_x is the number of molecules

5) O. Fukumoto, *J. Polymer Sci.*, **22**, 263 (1956).

6) K. Hoshino and M. Watanabe *J. Chem. Soc. Japan, Pure Chem. Sec.*, **70**, 170 (1949); *J. Am. Chem. Soc.*, **73**, 4816 (1951).

TABLE II
WEIGHT FRACTION OF OLIGOMERS DURING RANDOM DEGRADATION

Number-average degree of polymerization	Degree of degradation	Weight fraction of (%)			
		Monomer	Dimer	Trimer	Total
130					
65	0.0078	0.023	0.046	0.068	0.137
130/3	0.0155	0.054	0.105	0.154	0.313
26	0.0310	0.148	0.285	0.410	0.843
130/9	0.0620	0.479	0.892	1.245	2.616
	$p=0.9923$	Initial degree of polymerization 130			

composed of x monomer units, N is the total number of molecules and p is the extent of reaction. In a case where the bonds of all chains in the mixture at any given time are equally accessible to reaction independent of both their position in a chain and the length of their parent chains, the molecular weight distribution after degradation is given by

$$N_y(\alpha)=N[1-p(1-\alpha)]^2[p(1-\alpha)]^{y-1}(1-p)^{-1} \quad (2)$$

where $N_y(\alpha)$ is the number of molecules composed of y monomer units at the degree of degradation α. Substituting 1, 2, 3 etc. for y in equation (2), one can calculate the fraction of monomer, dimer, trimer etc. respectively at any degree of degradation. The results are summarized in Table II.

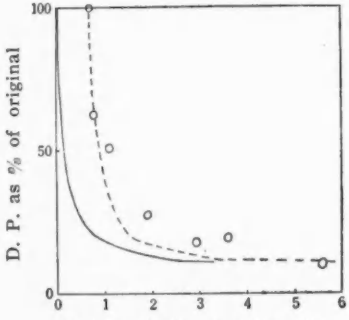

Fig. 4. Theoretical curve and observed relationship between the amount of water-soluble material and the number average degree of polymerization.
—— theoretical
- - - corrected
○ experimental

When the total weight fraction of monomer, dimer and trimer is plotted against number-average degree of polymerization, one can obtain the full line in Fig. 4. Observed values are represented by points. Taking it into account that a sample before degradation already has water-soluble material of 0.7%, one can reasonably say that the corrected value (dotted line) shows good agreement with the observed value.

Summary

1) The photochemical degradation of 6 nylon was investigated through the measurement of intrinsic viscosity and the amount of water-soluble material of 6 nylon taffeta irradiated for various periods of time in Fade-Ometer.

2) The intrinsic viscosity or the number-average degree of polymerization decreases rapidly at the initial stage, while the amount of water-soluble material increases approximately in proportion to the exposure time.

3) It is deduced from the relationship between the number-average degree of polymerization and the amount of water-soluble material that the molecular chain of 6 nylon is randomly split off by light. The relationship is well explained by the theory of random breaking of the polymer chain.

The author wishes to express his sincere thanks to Dr. K. Hoshino and Mr. M. Yoshida for their kind encouragement and permission to publish this paper.

Research Department Toyo Rayon Co., Ltd., Otsu, Shiga Prefecture

Spectrochemical Study of Microscopic Crystals. XVI[1]. *Structure of Cupric Formate, Acetate and Propionate*[2]

By Shoichiro YAMADA, Hiroji NAKAMURA and Ryutaro TSUCHIDA

(Received July 5, 1957)

On the basis of magnetic measurements, Bleaney and Bowers[3] supposed that in the crystal of cupric acetate monohydrate there were isolated pairs of copper ions coupled by exchange forces, with each copper ion bonded by four oxygen atoms in a plane. Van Niekerk and Schoening[4] examined the crystal structure of cupric acetate monohydrate and found that the compound in the crystalline state consists of dimeric molecules having a molecular formula of $Cu_2(CH_3COO)_4 \cdot 2H_2O$, in which a sort of direct interaction might exist between the copper ions, judging from the comparatively short copper-to-copper distance. The nature of the possible copper-to-copper interaction, however, was not completely clarified by those studies. It seems significant to examine this point on the basis of other properties. In order to study the above problem in more detail and to find, if possible, some correlation between the light absorption and the possible metal-to-metal interaction, we have determined dichroism of cupric formate tetrahydrate, cupric acetate monohydrate, anhydrous cupric propionate and its monohydrate.

Experimental

Materials.—Cupric formate tetrahydrate was prepared from cupric carbonate and formic acid. Blue crystals belonging to the monoclinic system are usually obtained in rectangular plates with a well developed (001)-plane. The compound is soluble in water, but insoluble in organic solvents. The measurement of dichroism was made on the (001)-plane with polarized light having its electric vector along the *b*- and the *a*-axis[5]. The *b*- and the *a*-absorption represent the absorption with the electric vector along the *b*- and the *a*-axis, respectively.

Cupric acetate monohydrate of reagent grade was recrystallized from 50% ethanol. Bluish green crystals were usually obtained in monoclinic plates with the (001)- or the $(20\bar{1})$-plane well-developed[5]. The compound is soluble in water and ethanol, but insoluble in chloroform. The measurement of dichroism was made on the $(20\bar{1})$-plane with polarized light having its electric vector along and perpendicular to the *b*-axis. The *b*- and the *a*-absorption represent the absorption with the electric vector along and perpendicular to the *b*-axis.

Cupric propionate monohydrate was prepared from cupric hydroxide and propionic acid. Recrystallization from ethanol-water mixture yielded bluish green crystals having an empirical formula of $Cu(C_2H_5COO)_2 \cdot H_2O$. The measurement of dichroism was made on one of the well-developed planes[6].

Anhydrous cupric propionate was obtained in bluish green crystals by repeated recrystallization of the cupric propionate monohydrate from chloroform. The color of the anhydrous compound is similar to that of the monohydrate. The absence of water of crystallization was readily confirmed by its infra-red spectrum. The measurement of dichroism was carried out on a well-developed plane. Anhydrous cupric propionate is almost insoluble in water, but is soluble in organic solvents such as ethanol and chloroform.

Measurement. — Quantitative dichroism measurement in the visible and the ultra-violet region was made by Tsuchida-Kobayashi's microscopic method[7].

Absorption spectra of the compounds in ethanol or chloroform were determined with a Beckman DU spectrophotometer. When a solution is too dilute, Beer's law is not obeyed. Even in such a dilute solution, however, Beer's law holds in the presence of an excess of the free aliphatic carboxylic acid. When ethanol was used as a solvent, it was apt to form some amount of ethyl carboxylate with the free ligand which was added in excess. The measurement, therefore, was made as quickly as possible after the solution was prepared. In any case, the obedience of the solution to Beer's law was always confirmed in the presence of free ligand.

1) Part XV of this series, S. Yamada and R. Tsuchida, This Bulletin, **30**, 715 (1957).

2) A brief summary of the present article was published as a letter to the editor: R. Tsuchida and S. Yamada, *Nature*, **176**, 1171 (1955).

3) B. Bleaney and K. D. Bowers, *Proc. Roy. Soc.*, **A214**, 451 (1952).

4) J. N. van Niekerk and F. R. L. Schoening, *Acta Cryst.*, **6**, 227 (1953).

5) We are indebted to Dr. R. Kiriyama of the Chemistry Department for the identification of the planes.

6) P. Groth, "*Chemische Kristallographie*", part 3, Engelmann, Leipzig, 1910 p. 205.

7) R. Tsuchida and M. Kobayashi, "*The Colour and the Structure of Metallic Compounds*", Zoshindo, Osaka, 1944, p. 180. See also previous papers of the present series.

Absorption spectra of cupric formate and acetate in aqueous solution were found to be essentially similar to that of the aquated cupric ion and, therefore, are not reported here.

The notations in the present paper are the same as those used in the former papers of this series. Extinction coefficients, ε, are calculated on the basis of the empirical formula instead of the molecular formula.

Results and Discussion

Cupric Formate Tetrahydrate.— The results of the dichroism measurements are shown in Fig. 1. According to the

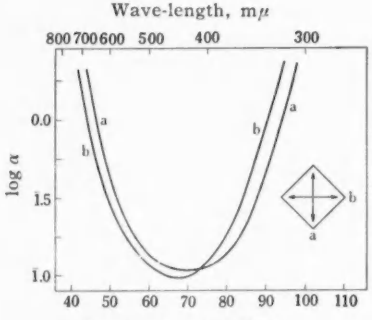

Fig. 1. Absorption spectra of cupric formate tetrahydrate.

crystal structure analysis[8], a copper ion is co-ordinated to four oxygen atoms in a plane of four formate ions, with two oxygen atoms of water molecules at a greater distance in the direction normal to the above plane. The crystal is found to show a band at about 38 to 39×10^{13}/sec. and a strong absorption band in the further ultra-violet region.

Typical compounds of copper(II) are usually known to show only one absorption band of moderate intensity in the near infrared to the near ultra-violet and another absorption band of much greater intensity in the further ultra-violet region. This is found to be the case with the cupric formate. Hence, it is suggested that no extraordinary linkage may be involved in the crystal of cupric formate tetrahydrate.

Although we could not determine the maximum of the band, the slope in the long wave-length region suggests the following relationship. For the band of the cupric formate at the longer wave-length, which is supposed to be closely related with the linkage between a copper ion and ligands, absorption is much stronger along the complex plane than along the direction normal to the plane. This is in good agreement with the relationship on the dichroism of planar cupric complexes of an ordinary type[9,10].

Cupric Acetate Monohydrate.—The results of the measurements are shown in Fig. 2 and Table I. The crystal structure

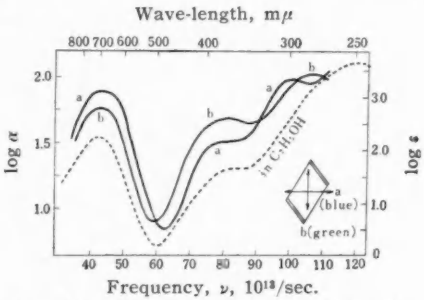

Fig. 2. Absorption spectra of cupric acetate monohydrate in the crystalline state (——), and in ethanol (----).

TABLE I
ABSORPTION MAXIMA OF CUPRIC ACETATE MONOHYDRATE IN THE CRYSTALLINE STATE

	band I		band II		band III	
	ν	log α	ν	log α	ν	log α
a-abs.	43	1.89	81	1.51	101	1.99
b-abs.		1.76		1.69	107	2.09

ν: 10^{13}/sec.

analysis of cupric acetate monohydrate was reported by van Niekerk and Schoening[4]. According to the structure analysis, the line joining the two copper ions within the dimeric molecule in the crystal makes an angle of 34.6° with the *ac*-plane and its projection onto the *ac*-plane an angle of 32.5° with the *c*-axis. Although the absorption spectra, which have been determined with the crystal, may represent the absorption spectra of the dimeric molecules under the crystalline field, we may approximately assume that the absorption spectra with the crystal represent the principal characteristics of the molecule itself. The intensity ratio of the Z-absorption to the X-absorption has been estimated from the present dichroism measurement and on the basis of the

8) R. Kiriyama, H. Ibamoto and K. Matsuo, *Acta Cryst.*, **7**, 482 (1954).

9) S. Yamada and R. Tsuchida, This Bulletin, **29**, 694, 289 (1956).

10) Idem, *Annual Rep. Scient. Works, Fac. Sci. Osaka Univ.*, **4**, 79 (1956).

structure analysis. For both the bands at 43 and 80×10^{13}/sec., the ratio has been found to be marked; *the ratio of K_z to K_x is nearly one tenth for the band at 43×10^{13}/sec., and about ten for the band at 80×10^{13}/sec.* The Z-absorption means the absorption with the electric vector along the line joining the two copper ions within a molecule, and the X-absorption, the absorption, with the electric vector along the plane which is normal to the *b*-axis (Fig. 3). It is assumed in the estimation that all the linkages between the copper and oxygen atoms are equivalent to one another.

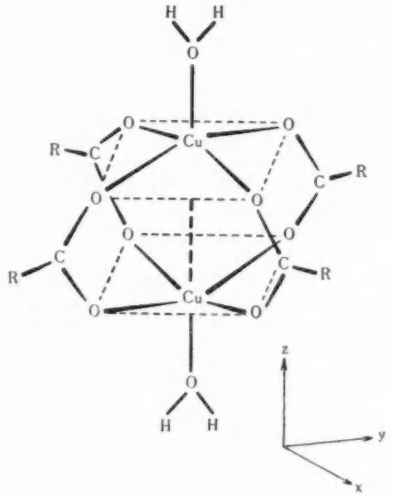

Fig. 3. Molecular structure of $Cu_2(R\cdot COO)_4\cdot 2H_2O$.

As was described in the preceding section, most cupric complexes of an ordinary type show only one band of moderate intensity in the region from the near infra red to the near ultra-violet. On the contrary, the present measurement indicates that cupric acetate monohydrate in the crystalline state shows two absorption bands at about 43 and 80×10^{13}/sec. (Fig. 2). The band at 43×10^{13}/sec. may apparently correspond to the so-called "copper band" which is characteristic of typical cupric complexes and which is supposed to be closely related with the linkages between the copper and the oxygen atoms. The band is usually considered as originating from the copper ion under the effect of the ligands. *For the band at 43×10^{13}/sec. absorption is found to be about ten times as strong with the electric vector along the complex plane as with the electric vector along the line joining the two copper ions within a molecule.* This is the same as the relationship found for the "copper band" of ordinary planar complexes of copper(II). These facts seem to support the above assignment of the absorption bands at about 43×10^{13}/sec. to the "copper band".

The absorption band at 80×10^{13}/sec. may be considered as an absorption band of a new kind for cupric complexes, judging from the wave-length and the intensity of the band. Therefore, the appearance of this band seems to suggest an extraordinary structure for this compound. This may be consistent with the hypothesis of the dimeric molecule with a formula of $Cu_2(CH_3COO)_4\cdot 2H_2O$.

Moreover, the polarization property with this new band at about 80×10^{13}/sec. is seen to be the reverse of the polarization with the band at 43×10^{13}/sec. *Thus for the band at about 80×10^{13}/sec. electric vector is absorbed much more strongly in the direction normal to the complex plane than along the plane.* The polarization with the band at 80×10^{13}/sec. may be understandable on the assumption of the copper-to-copper interaction, since the interaction may be expected in the direction normal to the complex plane. The band at about 80×10^{13}/sec. may be closely related with the copper-to-copper interaction.

The compound in the crystalline state shows an absorption band at about 100×10^{13}/sec. in addition to the two absorption bands. Although the origin of this band is not clear, we might assume the band to be due to the acetate groups in co-ordination. The band may well be called a kind of "specific absorption band" originating from the acetate ions in co-ordination. This band in solution is supposed to be hidden under a larger absorption band.

It is seen from Fig. 2 that cupric acetate in ethanol shows absorption bands at 43 and 80×10^{13}/sec., both with moderate intensity. French and Lowry[11] also reported absorption spectrum of cupric acetate in ethanol. The absorption curve, which they reported, agrees with ours in the longer wave-length, but differs from ours in the shorter wave-length. Thus they failed to find a band at about 80×10^{13}/sec. which has been clearly seen in our measurement. The appearance of the band at about 80×10^{13}/sec.,

11) H. S. French and T. M. Lowry, *Proc. Roy. Soc.*, **A 106**, 489 (1924).

as has been shown in the present measurement, may indicate that cupric acetate in ethanol involves similar components to those in the crystalline cupric acetate. It is supposed, therefore, that cupric acetate in alcohol consists of the dimeric molecules having a formula of $Cu_2(CH_3COO)_4 \cdot X_2$, X being the solvent molecule.

Summarizing the above discussion, we may conclude that *cupric acetate in organic solvents as well as in the crystalline state consists of dimeric molecules, in which a direct type of interaction may exist between the two copper(II) ions.*

Cupric Propionate Monohydrate.—The results of the measurements are shown in Fig. 4 and Table II. It is seen that

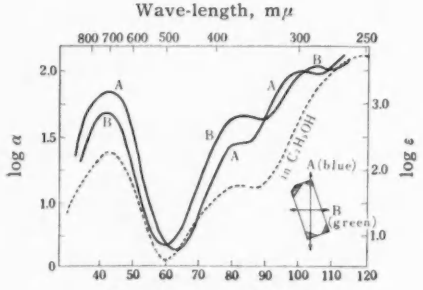

Fig. 4. Absorption spectra of cupric propionate monohydrate in the crystalline state (——), and in ethanol (----).

TABLE II
ABSORPTION MAXIMA OF CUPRIC PROPIONATE MONOHYDRATE IN THE CRYSTALLINE STATE

	band I		band II		band III	
	ν	$\log \alpha$	ν	$\log \alpha$	ν	$\log \alpha$
A-abs.	43	1.84	83	1.44	101	1.99
B-abs.		1.68		1.66	106	2.03

ν: 10^{13}/sec.

the absorption spectrum bears a close resemblance to the spectrum of cupric acetate. Thus cupric propionate in ethanol and chloroform and in the crystalline state exhibits two absorption bands at 43 and 80×10^{13}/sec. having almost the same intensity as the corresponding value of cupric acetate. As described in the above section, the appearance of the new absorption band at about 80×10^{13}/sec. may be regarded as showing the existence of the dimeric molecules.

Analysis of the crystal structure has not been done as yet. The present dichroism measurement, however, indicates that the polarization property with the band at about 80×10^{13}/sec. is the reverse of the polarization property with the band at about 43×10^{13}/sec. The reversal of the polarization property may readily be understood on the assumption of the presence of the dimeric molecule.

All the above facts seem to lead us to the conclusion that *cupric propionate monohydrate in organic solvents such as chloroform and ethanol and in the crystalline state may consist of the dimeric molecules with a formula of* $Cu_2(C_2H_5COO)_4 \cdot X_2$, *where X represents* OH_2 *or a molecule of the organic solvent.* This conclusion seems to be in agreement with the anomalous paramagnetic resonance absorption of cupric propionate monohydrate[12].

Anhydrous Cupric Propionate.—The dichroism of the anhydrous cupric propionate is shown in Fig. 5 and in the data given in Table III. The crystal structure

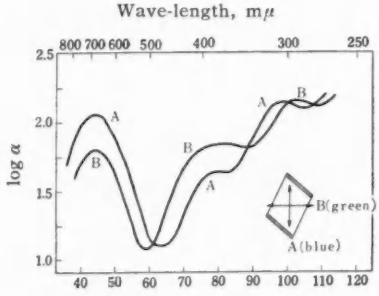

Fig. 5. Absorption spectra of anhydrous cupric propionate in the crystalline state.

TABLE III
ABSORPTION MAXIMA OF ANHYDROUS CUPRIC PROPIONATE IN THE CRYSTALLINE STATE

	band I		band II		band III	
	ν	$\log \alpha$	ν	$\log \alpha$	ν	$\log \alpha$
A-abs.	44	2.05	80	1.63	98	2.13
B-abs.		1.79		1.84	102	2.15

ν: 10^{13}/sec.

analysis of the anhydrous compound has not been reported as yet. But comparison of the dichroism of the anhydrous propionate with that of the monohydrate clearly indicates that the dichroisms for both compounds are essentially of an identical type, suggesting the existence of similar components in both the compounds. *Thus anhydrous cupric propionate in the crystalline*

12) H. Abe, *Phys. Rev.*, **92**, 1572 (1953).

state is supposed to consist of the dimeric molecules, in which a direct type of interaction may exist between the two copper ions.

It may also be concluded that *the water of crystallization has very little effect upon the dichroism*, and that *the linkage, if any, of the water molecules to the copper ion is not very strong in the crystal of cupric acetate monohydrate nor in cupric propionate monohydrate either.*

Direct Type of Interaction between the Copper Ions within a Molecule.—We have concluded in the preceding part of the present paper that cupric acetate and propionate in organic solvents and in the crystalline state consist of the dimeric molecules which involve a direct type of interaction between the two copper ions within the molecule. The absorption band at about 80×10^{13}/sec. may be related very closely with the direct interaction between the copper ions, although the nature of this band is not completely clear.

In previous papers we reported some examples in which a direct type of interaction might be expected between metallic ions[10,13]. In these compounds, there appeared, in the longer wave-length than the original absorption of their components, to exist a new type of absorption band polarized along the direction in which the metal-to-metal interaction was concluded. From this type of absorption bands the band at about 80×10^{13}/sec. of cupric acetate may differ, since the latter lies in the far shorter wave-length than the "copper band" which lies at 43×10^{13}/sec. We are inclined to suppose that the copper-to-copper interaction in cupric acetate may be akin to a kind of ordinary linkage. This seems to be compatible with the significantly shorter distance of 2.64Å between the metallic ions in the molecule of cupric acetate than the corresponding distance in Magnus's green salt or bis-(dimethylglyoximato)-nickel(II), in which the metal-to-metal interaction may be assumed to be weaker. The metal-to-metal distances in the latter two compounds were found to be 3.16 and 3.25 Å, respectively[14,15].

It would be worth noting that the intensity of the absorption band at about 43×10^{13}/sec. of cupric acetate and propionate in solution is considerably great, as compared with the intensity of the ordinary "copper band" for typical complexes of copper (II). This fact might have some relation with the unusual structure of the present cupric compounds.

13) S. Yamada, *J. Am. Chem. Soc.*, **73**, 1579 (1951); This Bulletin, **24**, 125 (1951); S. Yamada and R. Tsuchida, ibid., **27**, 156 (1954).
14) E. G. Cox, *J. Chem. Soc.*, **1932**, 1905.
15) L. E. Godycki and R. E. Rundle, *Acta Cryst.*, **6**, 487 (1953).

Summary

Dichroisms in the visible and the ultraviolet region have been determined by the microscopic method with cupric formate tetrahydrate, cupric acetate monohydrate, anhydrous cupric propionate and its monohydrate.

The cupric formate shows an absorption band at about $38-39\times10^{13}$/sec. which is considered to be due to the copper ion in combination with the ligands. For this band, electric vector is much more strongly absorbed along the plane of the complex than in the direction normal to the plane. The dichroism and the absorption spectrum of the cupric formate are of the same type as those of most cupric complexes involving no special effect upon the complexes.

Cupric acetate monohydrate, anhydrous cupric propionate and its monohydrate show an absorption band of a new kind at about 80×10^{13}/sec., in addition to a band at about 43×10^{13}/sec. which corresponds to the band at about 40×10^{13}/sec. of the cupric formate. The polarization for the band at 80×10^{13}/sec. is the reverse of the polarization for the band at 43×10^{13}/sec. From the appearance of the new band at 80×10^{13}/sec. and the reversal of the polarization property with this band, it has been concluded that the cupric compounds examined in this work, except the formate, consist of dimeric molecules having a structure similar to that of cupric acetate monohydrate.

It has also been shown that the water of crystallization has very little effect upon the linkages within the complex molecules. The cupric compounds, except the formate, show in organic solvents a band at 80×10^{13}/sec., involving the dimeric molecules in solution.

The present authors wish to thank Dr. R. Kiriyama and Dr. H. Abe for their interest in the present work. The work reported here was supported in part by a grant-in-aid from the Ministry of Education.

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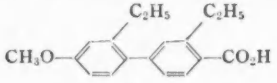
Estrogenic Biphenyls. IV. 3′-Alkyl-4-methoxybiphenyl-4′-carboxylic Acids

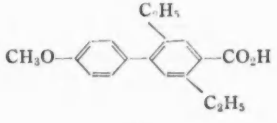
By Takeo SATO and Michinori ŌKI*

(Received July 22, 1957)

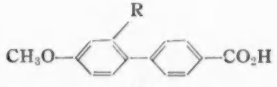
It has been found that 2,3′-diethyl-4-methoxybiphenyl-4′-carboxylic acid[1] (I) and 2′,5′-diethyl-4-methoxybiphenyl-4′-carboxylic acid[2] (II) are fully estrogenic to ovariectomized mice at the doses of 100 γ and that the enhanced estrogenic potency has been attributed to the presence of the two ethyl groups. In the previous paper[3], the role of the alkyl group of 2-alkyl-4-methoxybiphenyl-4′-carboxylic acids (III) in the estrogenic activity was studied and no significant difference was found in the activity of the members of this series.



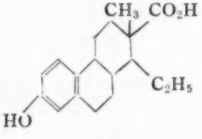
I



II



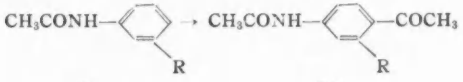
III



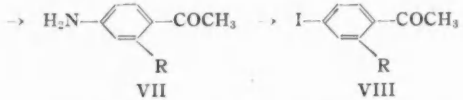
IV

Since these compounds are related structurally to doisynolic acid (IV), the effect of the alkyl group at position 3′ on the estrogenic activity is expected to be great[4]. Therefore the authors wished to find out the optimum alkyl group at position 3′ of 4-methoxybiphenyl-4′-carboxylic acid (III, R=H).

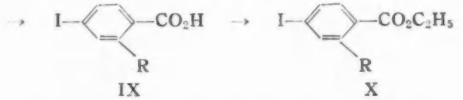
3′-Alkyl-4-methoxybiphenyl-4′-carboxylic acids (XII) were synthesized in the same way as compound I. All the compounds up to X where R is ethyl are known[1].



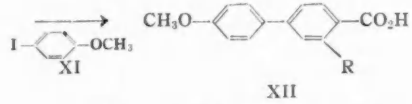
V VI



VII VIII



IX X



XI XII

a: CH_3 b: C_2H_5 c: n-C_3H_7

m-Alkylacetanilide (V) was treated with acetyl chloride and aluminium chloride to give 4-acetamido-2-alkylacetophenone (VI), which was hydrolyzed to 2-alkyl-4-aminoacetophenone (VII). Diazotization of compound VII and treatment of the diazonium salt with potassium iodide yielded 2-alkyl-4-iodoacetophenone (VIII), which was oxidized with sodium hypobromite to produce 2-alkyl-4-iodobenzoic acid (IX). The ethyl ester (X) was prepared in the usual way. Compound X and *p*-iodoanisole (XI) were submitted to the Ullmann reaction and the product was hydrolyzed to produce the desired compound (XII).

The structure of 2-alkyl-4-iodobenzoic acid (IX_a and IX_b) was proved in the following way. Compound IX gave a dibasic acid (XIII) on oxidation with potassium permanganate and the acid (XIII) gave known 4-iodophthalic anhydride (XIV) on sublimation and then 4-iodophthalimide (XV)[5] when the compound XIV was treated with ammonia.

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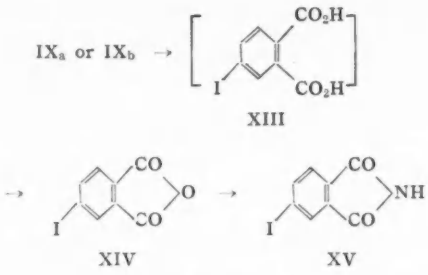
1) M. Ōki and T. Sato, This Bulletin, **30**, 508 (1957).
2) M. Ōki and T. Sato, ibid., **30**, 702 (1957).
3) T. Sato and M. Ōki, ibid., in press.
4) See K. Miescher, *Chem. Rev.*, **43**, 367 (1948).
5) A. Edinger, *J. prakt. Chem.*, (2), **53**, 375 (1896).



XIII XIV XV

TABLE I

ULTRAVIOLET ABSORPTION MAXIMA AND ESTROGENIC ACTIVITY OF 3'-ALKYL-4-METHOXYBIPHENYL-4'-CARBOXYLIC ACID (XII)

Compound	$\lambda_{max.}$ (mμ)	log $\varepsilon_{max.}$	Minimum active dose in mice (γ)
XII_a	287	4.39	500
XII_b	286	4.36	250
XII_c	286	4.38	500

The ultraviolet absorption data and the estrogenic activity of compounds XII_a, XII_b and XII_c are given in Table I. All the members of the present series showed a strong absorption at about 286 mμ (log ε =4.36), while 4-methoxybiphenyl-4'-carboxylic acid (III, R=H) at 289 mμ, as previously reported[3]. The spectra of compounds XII and III (R=H) were expected to resemble, since substitution at position 3' was regarded to have little effect on the conformation of the biphenyl skeleton. However, it may be noted that a hypsochromic shift of the absorption maxima has been observed rather than a bathochromic shift which is common when an alkyl group is introduced into the benzene ring. Similar results have been noted by other workers, when alkyl substitution takes place at the ortho position to the carboxyl group of the benzoic acid[6-8] and have been explained on the basis of the steric effect of the alkyl group which enforces the carbonyl group to rotate and to lose resonance with the benzene ring.

The minimum active doses of the members of the series XII to produce full estrus were determined by the vaginal smear test with ovariectomized mice. The alkyl substitution at position 3' enhances the estrogenic activity of 4-methoxybiphenyl-4'-carboxylic acid, probably because of the increasing width of the molecule. It is noted, however, that the kinds of alkyl group show no remarkable difference in the estrogenic activity. This may be attributed to the similar molecular thickness, as is shown by the ultraviolet absorption.

6) S. D. Ross, *J. Am. Chem. Soc.*, **70**, 4039 (1948).
7) E. A. Fehner, ibid., **72**, 1404 (1950).
8) C. M. Moser and A. J. Kohlenberg, *J. Chem. Soc.*, **1951**, 804.

Experimental[9]

***m*-Propylacetanilide** (V_c). — *m*-Propylaniline[3] was treated with acetic anhydride in the usual way. The oily product distilled at 165—169°/3 mm. and solidified on cooling. Recrystallization from aqueous ethanol gave colorless needles, m. p. 51—52°. Yield 96% of the theoretical.

Anal. Found: N, 7.79. Calcd. for $C_{11}H_{15}ON$: N, 7.90%.

4-Acetamido-2-methylacetophenone (VI_a). —To a well-stirred mixture of 45 g. (0.30 mole) of *m*-methylacetanilide (V_a)[10], 43 g. (0.55 mole) of acetyl chloride, and 240 ml. of carbon disulfide, 142 g. (1.1 moles) of aluminium chloride was added in small portions in one hour. The mixture was then heated under reflux for one hour and allowed to stand at room temperature for another hour. The supernatant carbon disulfide layer was decanted off and the lower layer was poured into a mixture of cracked ice and hydrochloric acid. The precipitate was collected and used for the subsequent reaction without purification. The analytical sample was obtained by recrystallization from aqueous ethanol. Colorless needles, m. p. 135—136°.

Anal. Found: N, 7.40. Calcd. for $C_{11}H_{13}O_2N$: N, 7.33%.

4-Amino-2-methylacetophenone (VII_a). —A mixture of the crude VI_a and 300 ml. of dilute hydrochloric acid (1 : 1) was heated under reflux for two hours, and, after cooling, basified with aqueous sodium hydroxide. The organic material was extracted with ether and the ethereal extract was dried over potassium carbonate and concentrated to give crystals, which were recrystallized from benzene-petroleum ether. Pale yellow plates, m. p. 94—95°. Yield 29 g. or 64% of the theoretical based on the *m*-methylacetanilide.

Anal. Found: N, 9.18. Calcd. for $C_9H_{11}ON$: N, 9.15%.

4-Amino-2-propylacetophenone (VII_c). — Treatment of 140 g. (0.79 mole) of compound V_c with 70 g. (0.89 mole) of acetyl chloride and 300 g. (2.26 moles) of aluminium chloride in 700 ml. of carbon disulfide yielded a viscous oil. The mixture was extracted with benzene and the extract was heated under reflux with 500 ml. of hydrochloric acid (1 : 1) for two hours. The aqueous layer was separated and basified with sodium hydroxide. The base was extracted with ether and the extract was fractionated. Repeated fractionation gave an oil boiling at 155°/2 mm. which solidified on standing. The analytical sample was obtained by recrystallization from benzene-petroleum ether. Colorless needles, m.

9) All melting and boiling points are uncorrected.
10) F. Beilstein and Al Kuhlberg, *Ann.*, **156**, 66 (1870).

p. 77.5—79°. Yield 53 g. or 38% of the theoretical.

Anal. Found: N, 7.88. Calcd. for $C_{11}H_{15}ON$: N, 7.90%.

The acetyl compound (VII_c) was obtained in the usual way. Colorless plates, m. p. 95.5—96°, were obtained on recrystallization from aqueous ethanol.

Anal. Found: N, 6.74. Calcd. for $C_{13}H_{17}O_2N$: N, 6.39%.

2-Alkyl-4-iodoacetophenones (VIII). — The general procedure is described by an example of the preparation of 4-iodo-2-methylacetophenone. The diazonium salt solution prepared from 30 g. (0.2 mole) of compound VII_a, dilute sulfuric acid (42 g. or 0.42 mole of sulfuric acid and 100 ml. of water) and 14 g. (0.2 mole) of sodium nitrite in 30 ml. of water, was slowly added to a solution of 50 g. (0.3 mole) of potassium iodide in 70 ml. of water. Stirring was continued for thirty minutes at a low temperature and then at 50—60° for two hours. The mixture was extracted with ether and the ethereal extract was washed with aqueous sodium hydroxide, aqueous sodium thiosulfate, and water. 4-Iodo-2-methylacetophenone boiled at 134—138°/4 mm. and solidified on long standing. The analytical sample was obtained by recrystallization from petroleum ether. Colorless prisms, m. p. 23—24°. Yield 27 g. or 52% of the theoretical.

Anal. Found: C, 41.40; H, 3.13. Calcd. for C_9H_9OI: C, 41.56; H, 3.49%.

4-Iodo-2-propylacetophenone ($VIII_c$) was obtained in 51% yield from the corresponding amine (VII_c). Brown oil, b. p. 158—159°/8 mm. Since the purification of this compound was a tedious process, the crude product was used for the subsequent reaction without purification.

2,4-Dinitrophenylhydrazone of 4-Iodo-2-methylacetophenone ($VIII_a$). — Recrystallized from ethanol-ethyl acetate. Orange plates, m. p. 167—168°.

Anal. Found: N, 12.50. Calcd. for $C_{15}H_{13}O_4N_4I$: N, 12.73%.

2,4-Dinitrophenylhydrazone of 4-Iodo-2-propylacetophenone ($VIII_c$). — Recrystallized from ethanol-ethyl acetate. Deep-orange plates, m. p. 160—161°.

Anal. Found: N, 11.86. Calcd. for $C_{17}H_{17}O_4N_4I$: N, 11.97%.

2-Alkyl-4-iodobenzoic Acids (IX). — The general procedure is given by an example of the preparation of 4-iodo-2-methylbenzoic acid (IX_a) as follows. To a well stirred sodium hypobromite solution, prepared from 65 g. (0.36 mole) of bromine, 50 g. (1.25 moles) of sodium hydroxide and 250 ml. of water, a solution of 25 g. (0.096 mole) of compound $VIII_a$ in 130 ml. of dioxan was added in one hour. The temperature was kept below 10° during the addition and then at 50—60° for one hour. The excess of hypobromite was decomposed with sodium sulfite and the mixture was steam-distilled to remove bromoform. The remaining solution was acidified with hydrochloric acid and the product was recrystallized from aqueous ethanol. Colorless needles, m. p. 169—170.5°. Yield 20 g. or 79% of the theoretical.

Anal. Found: C, 36.74; H, 2.53. Calcd. for $C_8H_7O_2I$: C, 36.66; H, 2.69%.

4-Iodo-2-propylbenzoic acid (IX_c) was obtained in 80% yield. Colorless needles, m. p. 127—128°.

Anal. Found: C, 41.60; H, 3.50. Calcd. for $C_{10}H_{11}O_2I$: C, 41.41; H, 3.82%.

4-Iodophthalimide (XV). (A). From 4-Iodo-2-propylbenzoic Acid (IX_d).—A mixture of 1 g. of compound IX_d, 5 g. of potassium permanganate, and 80 ml. of water, was heated under reflux for eighteen hours. Sulfur dioxide was bubbled into the mixture and the resulting solution was extracted with ether for five hours. The ethereal extract was dried over anhydrous magnesium sulfate and evaporated. The residue was distilled and 800 mg. of pale yellow prisms, m. p. 111—112°, were obtained. A purer sample of 4-iodophthalic anhydride (XIV) was obtained by sublimation in vacuo. Colorless prisms, m. p. 114—116°. The reported melting point is 123°[5].

A mixture of the crude 4-iodophthalic anhydride (500 mg.) and 1 ml. of 28% aqueous ammonia was heated over a free flame to remove excess of ammonia and water and the residue was sublimed in vacuo. 4-Iodophthalimide was obtained in pale yellow plates melting at 226—228°. Yield 400 mg. or 80% of the theoretical. Reported melting point is 222—224°[5].

Anal. Found: N, 5.08. Calcd. for $C_8H_4O_2NI$: N, 5.13%.

(B) From 4-Iodo-2-methylbenzoic Acid (IX_a).—Compound IX_a was treated in the same way as described above. Pale yellow plates melting at 224—226° were obtained and showed no depression of the melting point when mixed with another specimen of 4-iodophthalimide described above.

Ethyl 2-Alkyl-4-iodobenzoates (X).—The general procedure is exemplified by the preparation of ethyl 4-iodo-2-methylbenzoate (X_a) as follows. A solution of 20 g. of compound IX_a in 200 ml. of ethanol was saturated with hydrogen chloride and the mixture was heated under reflux for six hours. The solution was concentrated and diluted with water. Extraction with ether followed by fractionation gave the pure ester boiling at 141—144°/4 mm. Yield 17 g. or 78% of the theoretical.

Anal. Found: C, 41.87; H, 4.07. Calcd. for $C_{10}H_{11}O_2I$: C, 41.40; H, 3,82%.

Ethyl 2-ethyl-4-iodobenzoate (X_b) was prepared as described elsewhere[1]. Ethyl 4-iodo-2-propylbenzoate (X_c) was obtained in 77% yield. B. p. 179—182°/16 mm.

Anal. Found: C, 45.72; H, 4.74. Calcd. for $C_{12}H_{15}O_2I$: C, 45.30; H, 4.75%.

3'-Alkyl-4-methoxybiphenyl-4'-carboxylic Acids (XII).—The general procedure of synthesis was illustrated by the following example. A mixture of 18.6 g. (0.079 mole) of *p*-iodoanisole (XI)[11] and 10.0 g. (0.035 mole) of compound X_a, was heated at 220—228° with stirring and 30 g. (0.47 atom) of activated copper bronze[12] was

11) M. P. Brenans, *Bull. soc. chim. France*, (3), **25**, 819 (1901).

12) E. C. Kleiderer and R. Adams, *J. Am. Chem. Soc.*, **55**, 4291 (1933).

added over a period of twenty-five minutes. The temperature was kept at 220—230° for ten minutes and then at 280° for forty minutes. The reaction mixture was extracted with acetone and the solvent was evaporated. The residue was refluxed with a mixture of 100 ml. of 10% aqueous sodium hydroxide and 200 ml. of ethanol for six hours. The mixture was concentrated and the residue diluted with water. Insoluble material was removed by filtration and the filtrate was acidified with hydrochloric acid. The crystalline material was collected and boiled with benzene. Removal of the insoluble material followed by concentration gave the desired product. 4-Methoxy-3'-methylbiphenyl-4'-carboxylic acid (XII_a) was obtained in colorless needles, m.p. 199.5—200°, on recrystallization from aqueous ethanol. Yield 1.0 g. or 12% of the theoretical.

Anal. Found: C, 74.35; H, 5.55. Calcd. for $C_{15}H_{14}O_3$: C, 74.36; H, 5.83%.

3'-Ethyl-4-methoxybiphenyl-4'-carboxylic acid (XII_b) was obtained in 15% yield. Colorless needles, m. p. 182—183°.

Anal. Found: C, 74.82; H, 6.57. Calcd. for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29%.

4-Methoxy-3'-propylbiphenyl-4'-carboxylic acid (XII_c) was obtained in 16% yield. Colorless needles, m. p. 170.5—171°.

Anal. Found: C, 75.85; H, 6.63. Calcd. for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71%.

Ultraviolet Absorption Spectra. — These were measured by using Hitachi Photo-electric Spectrophotometer Model EPU-2. Substances were dissolved in 95% ethanol.

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Studies on Polyelectrolytes XXXVIII. Polyion in Simple Salt Solution

By Mitsuru NAGASAWA and Ikumi KAGAWA

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The study of electrical potential and counter ion distributions around a polyion in simple salt solution is one of the most important for clarifying the abnormal behavior of polyion. In this field, several interesting theories have so far been reported, including, Hermans and Overbeek's (H. O. theory)[1], Kimball, Cutler and Samelson's (K. C. S.)[2], Oosawa, Imai and Kagawa's (O. I. K.)[3], and Wall and Berkowitz's (W. B.)[4]. Although almost the same model of polyion was used in these theories, different potential distribution curves and free energies of the system were obtained because of the difference in procedure. Calculated from their expressions of the potential distribution and the free energy, therefore, the volume of polyion differs considerably, and, moreover, all volumes thus calculated as well as that calculated from assuming the balance between osmotic pressure and Kuhn entropy force by Flory[5] are much larger than the volume experimentally estimated[6-8]. The disagreement between observed and calculated volumes has sometimes been explained by presuming an association of counter ion with polyion and sometimes by pointing out the failure of the Debye-Hückel approximation for the exponentials in Poisson-Boltzmann equation.

The present paper is offered for the purpose of making a contribution toward the comprehension of the relationship among the theories so far published and an understanding of the cause of disagreement between theories and experiments. Taking into account the differences

1) J. J. Hermans and Th. G. Overbeek, *Rec. trav. chim.*, **67**, 761 (1948).
2) G. E. Kimball, M. Cutler and H. Samelson, *J. Phys. Chem.*, **56**, 57 (1952).
3) F. Oosawa, N. Imai and I. Kagawa, *J. Polymer Sci.*, **13**, 93 (1954).
4) F. T. Wall and J. Berkowitz, *J. Chem. Phys.*, **26**, 114 (1957).
5) P. F. Flory and I. E. Osterheld, ibid., **58**, 653 (1954).
6) N. S. Schneider and P. Doty, ibid., **58**, 762 (1954).
7) H. J. L. Trap and J. J. Hermans, ibid., **58**, 757 (1954).
8) H. Fujita, M. Mitsuhashi and T. Homma, *J. Colloid Sci.*, **9**, 466 (1954).

observed in several divisions of their theories, the authors' discussion is centered around the following three points: First, the relationship among the theories of H.O., K.C.S. and O.I.K. is compared and, based on the comparison, the authors introduce an analytical equation of potential distribution which is thought to be more reasonable in this category of polyelectrolyte theory. The characteristic features of the potential curve thus obtained are described in detail in relation to the Donnan membrane potential. Second, it is shown that when this potential distribution curve is used, the electrical free energy of the system can be easily calculated by simultaneously charging all ions in the solution from the non-charged state to the real state. The free energy thus obtained is compared with those of other theories[1,3,9]. Third, the polyion volume is calculated on the usual assumption that the electrical repulsive force is equal to the Kuhn entropy force. The volume obtained is still much larger than the volume estimated from the viscosity data, despite the fact that it must be the smallest of all theoretical volumes. Some comments are made on the cause of this.

The model of polyion employed here is a sphere having a uniform charge density throughout. It is assumed that the polyion is in an infinitely large volume of uni-uni valent simple electrolyte solution, and also that it has negatively charged groups and that the counter ion is the same univalent ion as the positive ion of the added electrolyte.

Potential and Ionic Distribution Around Polyion

Let us consider that R denotes the radius of polyion sphere and Ze the fixed charge of the polyion, then the density of fixed charge in the polyion ρ_0 is given by

$$\rho_0 = Ze/\frac{4}{3}\pi R^3 \tag{1}$$

If it is assumed that we can express the relationship among this charge density (ρ_0), the concentration of the simple electrolyte in the solvent phase (n_c) and the concentration in the polyion sphere phase (n_c^P) by Donnan equilibrium, it follows that

$$n_c^P(\rho_0/e+n_c^P)=n_c^2 \tag{2}$$

and also that the potential difference between the polyion phase and the solvent phase (E_D) can be given by the following Donnan membrane potential equation:

$$E_D=-\frac{kT}{e}\ln\frac{\rho_0/e+n_c^P}{n_c}=-\frac{kT}{e}\ln\frac{n_c}{n_c^P} \tag{3}$$

Substitution of n_c^P calculated from Eq. (1) into Eq. (2) yields

$$E_D=-\frac{kT}{e}\ln\frac{1+\sqrt{1+\left(\frac{8\pi}{3}\frac{1}{Z}R^3n_c\right)^2}}{\left(\frac{8\pi}{3}\frac{1}{Z}R^3n_c\right)} \tag{4}$$

from which it follows that

$$E_D^\circ=-\frac{kT}{e}\frac{1}{\left(\frac{8\pi}{3}\frac{1}{Z}R^3n_c\right)}=\frac{kT}{e}\frac{\rho_0/e}{2n_c} \tag{5}$$

when $n_c \gg 2\rho_0/e$, i.e., $\frac{8\pi}{3}\frac{1}{Z}R^3n_c \gg 1$.

When E_D or E_D° is used, the potential distribution curves for a polyion at infinite dilution in H.O., K.C.S. and O.I.K. theory are simplified as follows:

H.O.,

$$\psi_1=E_D^\circ\left\{1-e^{-\kappa R}(1+\kappa R)\frac{\sinh(\kappa r)}{\kappa r}\right\} \qquad (r<R) \tag{6}$$

$$\psi_2=\frac{1}{2}E_D^\circ\{(1+\kappa R)e^{-\kappa R}-(1-\kappa R)e^{\kappa R}\}\frac{e^{-\kappa r}}{\kappa r} \qquad (r>R) \tag{7}$$

K.C.S., (1) When Donnan approximation is applied:

$$\psi_1=\frac{kT}{e}\sinh^{-1}\frac{e}{kT}E_D^\circ=E_D \tag{8}$$

$$\psi_2=0 \tag{9}$$

(2) When $e\psi/kT \ll 1$:
Both ψ_1 and ψ_2 are the same as Eqs. (6) and (7).

O.I.K.,

$$\psi_1=E_D\left\{1-\frac{2}{e^{\kappa R}+e^{-\kappa R}}\frac{\sinh(\kappa r)}{\kappa r}\right\} \tag{10}$$

Here, in the theory of K.C.S. the equations for uniform distribution of fixed charge only are considered and in the theory of O.I.K. the equation at infinite dilution of polyion only is considered, κ being the reciprocal thickness of ionic atmosphere, i.e.,

$$\kappa^2=\frac{8\pi e^2}{DkT}n_c \tag{11}$$

9) S. Lifson and A. Katchalsky, *J. Polymer Sci.*, **13**, 43 (1954).

In all three theories it can be easily observed that the potential ψ_1 becomes E_D or E_D° and ψ_2 becomes zero when R is very large, that is

$$\left.\begin{array}{l}\text{H.O., } \psi_1=E_D^\circ \\ \text{K.C.S.,} \\ \quad\text{(1) for Donnan approximation} \\ \qquad \psi_1=E_D \\ \quad\text{(2) for } e\psi/kT\ll 1 \qquad \psi_1=E_D^\circ \\ \text{O.I.K., } \psi_1=E_D\end{array}\right\} \quad (12)$$

This fact suggests a very close relationship among these three theories and the simple Donnan theory of ionic distribution, which is later discussed in more detail. (see p. 966)

The theory of Oosawa, Imai and Kagawa distingushes itself from the other theories in the feature that no Debye-Hückel approximation is used in its solution to the Poisson-Boltzmann equation. The fact that E_D, instead of E_D°, appears in the potential equation, Eq. (10) of this theory manifests this feature. Another difference observed between these three theories is the difference between the factors of the second term in the ψ_1 equations. This difference comes from the fact that in the O.I.K. theory the effect of the ionic distribution outside the polymer sphere upon the potential distribution inside is entirely neglected, while in the H.O. and the K.C.S. theory that effect is taken into account.

An inspection of the two differences among H.O., K.C.S. and O.I.K. theory may lead us to expect the following equations to be a better approximation in this category of the polyelectrolyte theory:

$$\psi_1=E_D\left\{1-e^{-\kappa R}(1+\kappa R)\frac{\sinh(\kappa r)}{\kappa r}\right\} \quad (r<R) \quad (13)$$

$$\psi_2=\frac{1}{2}E_D\{(1+\kappa R)e^{-\kappa R}-(1-\kappa R)e^{\kappa R}\}\frac{e^{-\kappa r}}{\kappa r} \quad (r>R) \quad (14)$$

This result can be easily verified through the following calculation: eqs. (13) and (14) can be easily calculated from the same fundamental equations as used by Hermans and Overbeek if the assumption $e(E_D-\psi_1)\ll 1$ instead of $e\psi_1/kT\ll 1$ is used. That is,

$$\Delta\psi_1=-\frac{4\pi}{D}[-\rho_0+n_c e\exp(-e\psi_1/kT) -n_c e\exp(e\psi_1/kT)] \quad (15)$$

$$\Delta\psi_2=-\frac{4\pi}{D}[n_c e\exp(-e\psi_2/kT) -n_c e\exp(e\psi_2/kT)] \quad (16)$$

As ψ_2 is in general smaller than kT/e, the Debye and Hückel approximation can be used for the exponentials in Eq. (16) and hence from that equation it follows that

$$\psi_2=A_2\frac{e^{-\kappa r}}{r}+A_2'\frac{e^{\kappa r}}{r} \quad (17)$$

where A_2 and A_2' are integral constants. However, ψ_1 may sometimes become considerably great, but even in such a case the difference between E_D and ψ_1, i.e., $\psi_1'=E_D-\psi_1$, may be so small that the linear approximation of the exponentials of $e\psi_1'/kT$ can be applied (see Fig. 1).

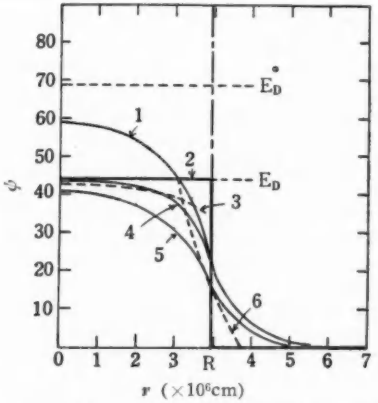

Fig. 1. Comparison of theoretical equations.
Sodium polyvinyl sulfate
$Z=1070$, $R=3.94\times10^{-6}$ cm
NaCl concn. $=1.31\times10^{-3}$ N
1. H.O.; 2. K.C.S.; 3. O.I.K.
4. Eqs. (13') and (14')
5. Eqs. (13) and (14)
6. Donnan-Helmholtz model

(Experimentally, ψ_1 is often greater than $kT/e=25.7$ mV at 25°C, but it is seldom greater than 2 kT/e even in considerably diluted solutions of simple salt. The assumption $e\psi_1'/kT<1$, therefore, may be true for a very wide range of simple salt solution.) When $\psi_1=E_D-\psi_1'$ is inserted in Eq. (15), the series expansion of exponentials of $e\psi_1'/kT$ are applied and higher terms are neglected, the equation becomes

$$\Delta\psi_1'=\kappa^2\cosh(eE_D/kT)\psi_1'=\kappa'^2\psi_1' \quad (18)$$

from which it follows that

$$\psi_1=E_D-\psi_1' =E_D-A_1\frac{e^{-\kappa' r}}{r}-A_1'\frac{e^{\kappa' R}}{r} \quad (19)$$

where A_1 and A_1' are integral constants and $\kappa'^2=\kappa^2\cosh(eE_D/kT)$.

Based on the boundary conditions that ψ is everywhere finite, is zero at infinity and is continuous on the surface of the sphere, all constants in Eqs. (17) and (19) can be determined. The final equations thus obtained are as follows:

$$\psi_1=E_D\left[1-\frac{1+\kappa R}{\kappa'\cosh(\kappa' R)+\kappa\sinh(\kappa R)}\cdot\frac{\sinh(\kappa' r)}{\kappa' r}\right] \quad (13')^*$$

$$\psi_2=E_D\left[\frac{(1+\kappa' R)e^{-\kappa' R}-(1-\kappa' R)e^{\kappa' R}}{\kappa'(e^{-\kappa' R}+e^{\kappa' R})-\kappa(e^{-\kappa' R}-e^{\kappa' R})}\cdot e^{\kappa R}\right]\frac{e^{-\kappa r}}{r} \quad (14')^*$$

If we assume $\kappa'=\kappa$ in Eqs. (13') and (14'), we obtain Eqs. (13) and (14). As shown by Wall and Berkowitz in their numerical solution of Eqs. (15) and (16), the term E_D has a strong effect on ionic distribution, but the factor of the second term is less important. Therefore, to assume $\kappa'=\kappa$ in this stage, i.e., the assumption that E_D is not *very* large, scarecely lessens the applicability of the theoretical equations.

We have made a comparison between the results calculated from these theoretical equations for an experimental example in Fig. 1. It is observed that the values calculated from Eqs. (13) and (14) are the smallest of all calculated values. The difference between the values calculated from these equations and others grows smaller as the concentration of the simple salt solution increases.

The charge distribution around polyion can be calculated as follows. As the charge density ρ is given by the following equation,

$$4\pi r^2\cdot\rho=-\Delta\psi\cdot r^2 D \quad (20)$$

from this equation and Eqs. (13) and (14) it follows that

$$4\pi r^2\rho_1=D\cdot E_D e^{-\kappa R}(1+\kappa R)\kappa r\cdot\sinh(\kappa r) \quad (r<R) \quad (21)$$

$$4\pi r^2\rho_2=-\frac{DE_D}{2}\{(1+\kappa R)e^{-\kappa R}-(1-\kappa R)e^{\kappa R}\}(\kappa r)e^{-\kappa r} \quad (r>R) \quad (22)$$

The total amount of the free charge inside and outside the polyion sphere ($[q]_1$ and $[q]_2$) can be calculated from the following integrations:

$$[q]_1=\int_0^R 4\pi r^2\rho_1 dr \quad (23)$$

$$[q]_2=\int_R^\infty 4\pi r^2\rho_2 dr \quad (24)$$

Hence, carrying out these integrations by using Eqs. (21) and (22), we have

$$[q]_1=-[q]_2=D\cdot\frac{E_D}{2\kappa}(1+\kappa R)e^{-\kappa R}[(1+\kappa R)e^{-\kappa R}-(1-\kappa R)e^{\kappa R}] \quad (25)$$

In Fig. 2, the result calculated from Eqs. (21) and (22) for the same example as in Fig. 1 is shown.

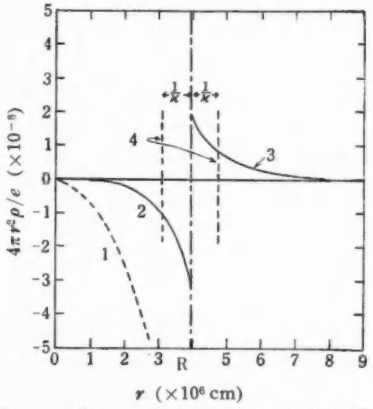

Fig. 2. Charge distribution calculated by Eqs. (21) and (22).
1. $4\pi r^2\rho_0$
2. $4\pi r^2\rho_1$
3. $4\pi r^2\rho_2$
4. Donnan-Helmholtz model

Calculation of the Electrical Free Energy

The electrical free energy discussed here means the difference between the free energy of systems at the real state and at the imaginary non-charged state, which causes the electrical repulsion of polyion sphere. That is, the electrical free energy is equal to the work which must be done when all ions in the solution are charged simultaneously from the imaginary non-charged state to the real state, keeping the interior and the exterior

* These equations agree entirely with those independently derived by Lifson[16]. He pointed out that these equations are very close to the numerical solution of Wall and Berkowitz. Eqs. (13') and (14') have a little wider applicability to experiments than Eqs. (13) and (14), but to calculate the free energy using them is very difficult. The order of applicability of various theoretical equations is as follows:

H. O.<Eqs. (13), (14)<Eqs. (13'), (14')<W. B.

In real experiments, however, experiments are rarely carried out in such a dilute simple salt solution as that in which the Hermans and Overbeek's theory does not hold, although Wall and Berkowitz have reported that H. O. theory gives far too high potential values.

16) S. Lifson, private communication.

of the polyion sphere at equilibrium. (This electrical free energy is not necessarily equal to that defined in the well-known theory of Debye and Hückel, where the work done while each ion is being charged against the electrical potential caused by the self-charge, must be subtracted from the electrical free energy defined here.)

The electrical free energy (F_e) of the system is given by the following integration:

$$F_e=\int_0^\infty\int_0^1\psi(\xi)\rho(\xi)/\xi 4\pi r^2\cdot dr\cdot d\xi$$
$$=\int_0^R\int_0^1\psi_1(\xi)\rho_1(\xi)/\xi\cdot 4\pi r^2\cdot dr\cdot d\xi$$
$$+\int_R^\infty\int_0^1\psi_2(\xi)\rho_2(\xi)/\xi\cdot 4\pi r^2\cdot dr\cdot d\xi \quad (26)$$

where ξ denotes the fraction of final charges which the ions have at any stage of the integration. After some simple calculations using Eqs. (13), (14), (21) and (22), F_e becomes

$$F_e=\frac{1}{2}D\frac{(\kappa R)^2}{2\kappa}E_D^2\int_0^{\kappa R}f(x)dx$$
$$=\frac{1}{2}D\frac{(\kappa R)^2}{2\kappa}E_D^2\sigma(\kappa R) \quad (27)$$

where

$$f(x)=\frac{(3+6x+5x^2+2x^3)e^{-2x}+(x^2-3)}{x^3} \quad (28)$$

$$\sigma(\kappa R)=\int_0^{\kappa R}f(x)dx \quad (29)$$

and the values of $\sigma(\kappa R)$ can be easily obtained by graphical integration.

Calculated Dimension of Polyion Sphere and a Comparison of Calculated and Experimental Dimensions

The dimension of polyion sphere may be calculated from the condition that the total free energy of the system, that is, the sum of the electrical free energy and the Kuhn entropy, must have the minimum value. That is,

$$\frac{d}{dR}[F_e+TS_K]=0 \quad (30)$$

Therefore,

$$dF_e/dR=-TdS_K/dR \quad (31)$$

The value of dF_e/dR is given by differentiating Eq. (27) as follows:

$$\frac{dF_e}{dR}=\frac{1}{2}D(\kappa R)E_D\left[E_D\left\{\sigma(\kappa R)+\frac{1}{2}(\kappa R)\frac{d\sigma(\kappa R)}{d(\kappa R)}\right\}+R\frac{dE_D}{dR}\sigma(\kappa R)\right] \quad (32)$$

Numerical values of $\sigma(\kappa R)$ and $d\sigma(\kappa R)/dR$ can be easily calculated by graphical method. In calculating the Kuhn entropy, we must express it as a function of R. Since such a calculation has already been performed by Hermans and Overbeek[10], we use their calculated result although it is a little different from the original Kuhn entropy; that is,

$$-T\frac{dS_K}{dR}=kT\left[\frac{3}{R^2-\frac{5}{36}NA^2}-\frac{108}{5}\frac{1}{NA^2}\right]\cdot R \quad (33)$$

where A is the dimension of statistical segment and N the number of segments in a polyion.

Thus, when we know the polymerization degree and the charge density, we can graphically calculate the value of R from Eqs. (30)–(33) by assuming a value of A.

The experimental value of R can be calculated from the data of intrinsic viscosity; that is, the end-to-end distance of polymer is first calculated based on Flory's theory[11], and next is transformed into the radius of polyion sphere. According to Flory, the intrinsic viscosity of a polymer is expressed as follows:

$$[\eta]=\frac{\Phi(\sqrt{h^2})^3}{M} \quad (34)$$

where M is the molecular weight of polymer and Φ is 2.1×10^{21} when $[\eta]$ is expressed by 100 c.c./g. The h is related to R by Hermans and Overbeek as follows:

$$R^2=\frac{5}{36}NA^2\left(1+\frac{h^2}{NA^2}\right) \quad (35)$$

The calculated and the experimental values of R thus obtained for polyvinyl alcohol sulfate are shown as an example in Table I. In this Table, in addition, the values of $-T(dS_K/dR)$ and (dF_e/dR) calculated by using the experimental values of R are shown. The value of A used for the calculation is 20–40 Å, which is thought to be reasonable for vinyl polymer[8].

It is observed that there is considerable disagreement between observed and calculated radii of polyion; values of the

10) D. T. F. Pals and J. J. Hermans, *Rec. trav. chim.*, **71**, 434 (1954).
11) P. F. Flory, "The Principles of Polymer Chemistry", Cornell University Press. p. 616 (1953).

TABLE
SODIUM POLYVINYL
Deg. of polym.=1470,

NaCl concn. in solvent (N)	$A=20$ Å			
	R_η	$R_{\text{calcd.}}$	$\left(-T\frac{dS_K}{dR}\right)_{R,\text{obs.}}$	$\left(\frac{dF_e}{dR}\right)_{R,\text{obs.}}$
1.00	1.73×10^{-6}	1.75×10^{-6}	1.3×10^{-7}	1.6×10^{-7}
5.00×10^{-1}	1.89	2.07	1.7	2.8
1.00	2.25	2.83	2·3	9.6
5.02×10^{-2}	2.41	3.34	2.5	1.7×10^{-6}
2.01	2.76	4.14	3.0	2.3
1.00	2.99	4.65	3.3	2.9
5.04×10^{-3}	3.31	5.60	3.7	2.7
2.02	3.69	6.75	4.2	1.2
1.31	3.81	8.25	4.4	(2.0×10^{-7})
8.09×10^{-4}	3.92	—	4.5	(-1.1×10^{-6})
4.00	4.50	—	5.2	(−1,3)

former are much lower than those of the latter, that is to say, $(dF_e/dR)_{R,\text{obs.}}$ is much larger than $-T(dS_K/dR)_{R,\text{obs.}}$. If we arbitarily choose the dimension A, we can find better agreement, but here the value of A becomes so great that it can not be recognized as reasonable. These facts agree with those reported by Fujita[8] based on the theory of Hermans and Overbeek[1]. The results calculated using another sample of lower polymerisation degree are the same as those mentioned above.

TABLE II
SODIUM CARBOXYMETHYL CELLULOSE
(by Pals and Hermans[10])
Deg. of Polym.=725, $Z=405$, $A=180$ Å

NaCl concn. in solvent (N)	R_η	$\left(-T\frac{dS_K}{dR}\right)_{R,\text{obs.}}$	$\left(\frac{dF_e}{dR}\right)_{R,\text{obs.}}$
∞	4.25×10^{-6}	0	0
0.192	4.31	1.3×10^{-9}	2.9×10^{-9}
0.096	4.39	6.1	9.0
0.048	4.53	1.3×10^{-8}	1.5×10^{-8}
0.024	4.66	1.7	4.5
0.012	4.83	2.4	6.5
0.006	5.07	3.1	2.0×10^{-7}
0.003	5.29	3.7	2.1
0.0015	5.57	4.5	3.5
0.00075	5.80	5.0	4.9
0.000375	6.11	5.6	4.5

In Table II, results calculated from data on carboxymethyl cellulose by Pals and Hermans[10] are given for reference. The value of A used for C.M.C. is 183 Å which is the same as used by Pals and Hermans. In this case also the calculated values are larger than the observed values. In section 2 it has been pointed out that the electrical potential ϕ calculated from Eq. (13) is the smallest of all the calculated potentials. As shown later, not only the electrical potential ϕ, but also the free energy equation used in this paper seem to give the smallest calculated values, and hence the values of R calculated from the present theory can be expected to be lower than those calculated from H.O. theory and others. Nevertheless, they are still much larger than the experimental values. Therefore, it is concluded that the improvement of Debye-Hückel linearization approximation is not sufficient to account for the discrepancy between calculated and experimental volumes of the polyion. The cause of the disagreement is discussed in the following section.

Discussion

a) **The relationship between the present theory and the Donnan approximation.**—From the discussion in section 2, it has been pointed out that theories based on the uniform sphere model have very close relationship with the Donnan theory. Here let us look over the details of the relationship. The survey may help us to understand the character of the theories.

At the Donnan membrane equilibrium, the potential difference between polyion sphere and outer solution E_D is expressed by Eq. (3). The existence of the potential difference means the existence of free charges at the boundary of two phases. In considering the charge distribution to be expressed by Helmholtz double layer model (see Fig. 3) and expressing the free charge by $[q]$ and the capacity of the double layer by K, there must be a relationship between $[q]$, K and E_D, as follows:

I
SULFATE
$Z=1070$

$A=40$ Å

R_7	$R_{\text{calcd.}}$	$\left(-T\frac{dS_K}{dR}\right)_{R,\text{obs.}}$	$\left(\frac{dF_e}{dR}\right)_{R,\text{obs.}}$
2.00×10^{-6}	2.15×10^{-6}	3.7×10^{-8}	8.0×10^{-8}
2.60	2.50	5.6	1.5×10^{-7}
2.46	3.40	9.3	5·6
2.61	3.85	1.1×10^{-7}	1.2×10^{-6}
2.94	4.75	1.4	1.9
3.17	5.30	1.6	2.5
3.46	6.35	1.8	2.7
3.81	7.65	2.1	1.4
3.94	—	2.2	4.6×10^{-7}
4.07	—	2.2	(-8.1×10^{-7})
4.60	—	2.6	(-1.3×10^{-6})

$$-[q]=KE_D \tag{36}$$

As these free charges constitute a sphere condenser, the electrical capacity K is expressed by

$$K=D\frac{R^2-\frac{\delta^2}{4}}{\delta}\fallingdotseq D\cdot\frac{R^2}{\delta} \tag{37}$$

where δ is the thickness of the double layer. When R is sufficiently great, δ can be assumed to be equal to $2/\kappa$. From Eqs. (36) and (37), it follows that

$$[q]\fallingdotseq -D\frac{(\kappa R)^2}{2\kappa}E_D \tag{38}$$

which agrees with Eq. (25) when κR is greater than unity, (If the effect of free charges in the solution phase be neglected that is, if it is assumed that $K=D\cdot R$, the surface charge of Oosawa, Imai and Kagawa can be obtained.)

From these considerations it is concluded that the Donnan model corresponds to the ion distribution which would have been obtained if the free ions distributed inside and outside the polymer according, respectively, to Eqs. (21) and (22) were rearranged to concentrate at the center of gravity of their respective distribution curves, as shown schematically in Fig. 2. The potential distribution corresponding to the model is shown in Fig. 1. When κ is sufficiently great, we can find no difference between the H. O. or the present theory and the Donnan theory. If not however, E_D does not equal E_D^0, but even in that case the potential and the charge distribution of the present paper can completely agree with that by the Donnan theory when R is very great (see Fig. 1).

b) **Comparison between the electrical free energies described in the present paper and in others.**—If we assume the model shown in Fig. 3, we can easily

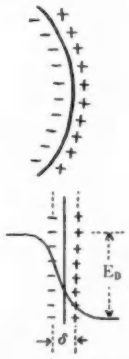

Fig. 3. Helmholtz double layer model.

calculate the electrical free energy of the system by the well-known theory of electrocapillarity[12]. That is,

$$\frac{dF_e}{dE}=-[q]=D\frac{(\kappa R)^2}{2\kappa}E_D \tag{39}$$

By integrating this equation at $R=$const., we have

$$F_e=\frac{1}{2}\frac{(\kappa R)^2}{2\kappa}E_D \tag{40}$$

By comparing this equation with Eq. (27), we can know that the charge distribution through the sphere results in the appearance of $\sigma(\kappa R)$.

In addition to these calculations, there are several others published on the electrical free energy: Hermans and Overbeek[1], Lifson and Katchalsky[9], and

12) J. Frenkel, "Kinetic Theory of Liquids", Oxford University press. p. 361 (1946).

Oosawa, Imai and Kagawa[3]. Hermans and Overbeek have given the following equation:

$$F_e(\text{H.O.}) = \frac{1}{2}\int_0^\infty \rho_0\psi \cdot 4\pi r^2 \mathrm{d}r \qquad (41)$$

where

$$\rho_0 = Ze/\frac{4}{3}\pi R^3 \qquad (42)$$

As can be understood from the physical meaning of this equation given by Hermans and Overbeek, their electrical free energy may equal the work done during the time when all charged groups of polyion are placed one-by-one on a non-charged skeleton of the polyion in simple salt solution, not taking into account the presence of counter ions. That is,

$$F_e(\text{H.O.}) = \int_0^R \int_0^Z \frac{e\psi}{\frac{3}{4}\pi R^3} 4\pi r^2 \cdot \mathrm{d}Z \cdot \mathrm{d}r \qquad (43)$$

It can be proved that this integration give the same result as Eq. (41). Considering the features of Eqs. (26) and (43), therefore, we can see that

$$F_e(\text{H.O.}) > F_e(\text{Eq.}(27))$$

According to Lifson and Katchalsky, the electrical free energy is given by the following equation:

$$F_e(\text{L.K.}) = \int_0^1 \frac{U(\xi)}{\xi}\,\mathrm{d}\xi \qquad (44)$$

where

$$U = \frac{D}{8\pi}\int_V (\text{grad}\,\psi)^2 \cdot \mathrm{d}V$$

Although it is too difficult to calculate this F_e using Eqs. (13) and (14), if the charge distribution be assumed to be expressed by the Donnan approximation, F_e can easily be calculated as follows:

$$F_e(\text{L.K.}) = \int_0^1 \frac{D}{8\pi}\frac{\kappa^2 \cdot E_D^2}{4} 4\pi R^2 \frac{2}{\xi\kappa}\frac{1}{\xi}\mathrm{d}\xi$$

$$= \frac{1}{2}D\frac{(\kappa R)^2}{2\kappa}E_D^2\int_{\kappa}^{1}\frac{1}{\xi^2}\mathrm{d}\xi \qquad (45)$$

because

$$\text{grad.}\ \psi(\xi) \fallingdotseq \frac{E_D(\xi)}{2\kappa(\xi)} = \frac{E_D \cdot \kappa}{2} \qquad (46)$$

Except for the difference between integral terms of Eqs. (45) and (40), both show the same result. (That the integral term of Eq. (45) becomes infinity at $\xi=0$ is because of the failure of the Donnan model.) We way now conclude that the electrical free energy of Eq. (26) may agree, at least qualitatively, with that of Lifson and Katchalsky.

The calculation of F_e used in the theory of Oosawa, Imai and Kagawa is not applicable to our system, in as much as the free volume is assumed in their theory. From numerical comparison, however, we can see that F_e of Eq. (27) may not have values higher than their free energy. As presumed by Flory[13], the osmotic pressure calculated from the number of simple ions gives almost the same value as our electrical repulsive force, but, strictly speaking, the former is a little higher than the latter. Thus, the electrical free energy calculated from Eq. (27) may be expected to be the smallest of all the calculated values.

c) On the cause of discrepancy observed between calculated and observed values.—When we consider the reason for the disagreement, we must first remember that the Donnan potential difference observed for macroscopic polyelectrolyte solution is usually much lower than the ideal value, Eq. (3)[14]. If the character of term E_D in Eqs. (13) and (14) be similar to that of the Donnan potential observed for macroscopic polyelectrolyte solution, the potential expressed by Eq. (13) may be much higher than the real potential, and hence the electrostatic repulsive force may have been overestimated in this paper. It is true that there is no reason why we should consider the ionic distribution around a polyion to be the same as that observed for the macroscopic system. Considering, however, the fact that the concentration of the counter ion within the polymer phase is almost the same as the concentration usually used for experiments of macroscopic Donnan equilibrium (for example, 8.19×10^{-2}—4.35×10^{-3}N for sodium polyvinylsulfate in Table I), it seems to be also true that there is no reason why we should not consider the term E_D in Eqs. (13) and (14) to be of the same character as the macroscopic Donnan potential. (The result that the calculated values are comparatively close to the observed ones in concentrated solutions of sodium chloride, agrees with the behavior of macroscopic Donnan membrane equilibrium.) Although for a complete elucidation of this phenomenon there must be

13) P. F. Flory, ibid., p. 631.
14) M. Nagasawa, H. Nakoji and I. Kagawa, *J. Chem. Soc. Japan, Ind. Chem. Soc.*, **57**, 9 (1954).

more intensive work, it seems certain that there are some important electrostatic effects other than those considered here. On further speculation we can name one of these effects which may be expressed in the following several ways:

(1) The association of the counter ion with polyion must be taken into account; this was pointed out by Flory and Osterheld[5].

(2) The "P" binding must occur in the polyion sphere, as reported by Oosawa, Imai and Kagawa[3].

(3) The activity coefficients of ions gathering within polyion sphere are very low compared with unity owing to their strong electrostatic interaction. In other words, the effect of the local ionic atmosphere around a simple ion must be taken into account.

(4) Schematically speaking, the fact that the polyion thread is enveloped by a double layer of cylindrical symmetry must be taken into account, as pointed out by Overbeek and Stigter (see Fig. 4)[15].

Fig. 4. Schematic picture of coiled polyelectrolyte chain.

It is to be noted that these expressions have the same meaning and give the same results of decrease in the value of the term E_D of potential distribution.

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15) J. Th. G. Overbeek and D. Stigter, *Rec. trav. chim.*, **75**, 543 (1956).

The Vapor Pressure of Germanium Dioxide

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Though germanium dioxide is expected to be more or less volatile at higher temperatures, the exact magnitude of its vapor pressure is not known. Moreover, the thermodynamic properties of the dioxide have been but little studied. To make a contribution to the chemistry of germanium, we have determined the vapor pressure of solid germanium dioxide; the measurements were made by Knudsen's effusion method over the temperature range from 1,040°C up to its melting point.

There is no evidence for the existence of gaseous germanium dioxide. The most important gas species over the solid dioxide around these temperatures is considered to be monoxide vapor[1]; so the vaporization can be regarded as the dissociation reaction,

$$GeO_2\,(c)^* = GeO\,(g.) + 1/2\ O_2\,(g.). \qquad (1)$$

The possibility of other reactions, for instance the dissociation into constituent elements, is excluded from the free energy consideration; and, as the non existence of solid monoxide has been discussed by so many investigators, there may be no room for it as a dissociation product.

Experimental

Apparatus and Procedure.—The apparatus was the same as that used in the previous investigations carried out in this laboratory; the measuring technique was slihgtly modified as described elsewhere[2]. The weight of vapor effusing through an orifice out from the quartz Knudsen cell is measured by means of a microthermobalance enclosed in the vacuum system. The vapor pressure is then calculated from the relation

$$p = \sqrt{\frac{2\pi RT}{M}} \cdot \frac{1}{A} \cdot \frac{G}{\tau} \qquad (2)$$

where G is the weight loss of the sample in duration of τ seconds, A the orifice area and M the molecular weight of the gas molecule.

1) L. Brewer *Chem. Rev.*, **52**, 62 (1953).
2) E. Shimazaki and T. Wada, This Bulletin, **29**, 294 (1956).
* In this paper, GeO_2(c) means crystalline germanium dioxide of hexagonal α-quartz structure.

In this investigation, the mean molecular weight of two gaseous components was taken as M, the germanium monoxide vapor being assumed to be unimolecular. Inserting the values of constants and of the mean molecular weight, together with $A=4.19\times10^{-3}$ cm^2, we obtain from equation (2)

$$p\ (\text{mmHg}) = 489.99\frac{G}{\tau}\sqrt{T}. \qquad (3)$$

The Sample.—The sample of germanium dioxide, stated 99.99% pure, was of the make of Deutsche Otavi Eisenbahngesellschaft, and was used without any further purification. Prior to the measurements, it was heated in the air above 1,000° for several hours to remove any absorbed gases or moisture. This microcrystalline white powder, as well as the residue remaining in the cell after the experiment, showed nothing but the pattern of germanium dioxide of hexagonal quartz structure in the X-ray diffraction studies.

The coincidence of the cell temperature with the thermometer reading was ensured by the fact that the sample held at the temperature just above its melting point (1,116°C) was in glassy appearance in the quartz cell, indicating that it had melted.

Results and Discussion

The results of the experiment are summarized in Table I and plotted in Fig. 1. By means of the method of least squares, the results are expressed by equation (4), corresponding to the straight line in the $\log p$ vs. $1/T$ plot in Fig. 1.

$$\log p_{\text{mm.}} = -\frac{25,517}{T} + 16.245 \qquad (4)$$

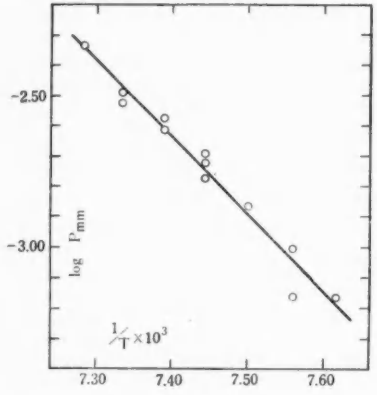

Fig. 1.

By differentitation of equation (4) with respect to $1/T$, or from the inclination of the straight line in Fig. 1, the heat of reaction (1) is obtained as

$$\Delta H_T = 116.7\pm0.2\ \text{kcal./mol.}$$

TABLE I

Tem. T°K	Loss in Weight $G\times10^3$ g.	Duration τ sec.	Vapor Pressure $p\times10^3$ mmHg.	$\log p+4$.
1,313	0.827	21,600	0.680	0.832
1,323	0.808	18,000	0.689	0.838
〃	1.000	〃	0.990	0.996
1,333	1.365	18,000	1.356	1.132
1,343	1.173	12,600	1.672	1.223
〃	2.038	18,000	2.033	1.308
〃	1.500	14,400	1.870	1.272
1,353	1.327	9,000	2.657	1.424
〃	1.923	14,400	2.407	1.381
1,363	2.384	14,400	2.995	1.476
〃	2.557	〃	3.212	1.506
1,373	2.769	10,800	4.655	1.668

The orifice area $A=4.19\times10^{-3}$ cm^2.

The free energy and the entropy change are also calculated,

$$\Delta G_T = 34.0\ \text{kcal./mol.}$$
$$\Delta S_T = 61.1\ \text{kcal./mol./deg.}$$

where underscript T denotes the mean temperature in the experiment. To construct the free energy equation, the following heat capacity equations were used:

GeO_2(c) [3] $\quad C_p = 11.2+7.17\times10^{-3}T$

O_2(g) [3] $\quad C_p = 7.16+1.00\times10^{-3}T - 0.40\times10^5T^{-2}$

GeO(g) $\quad C_p = \frac{7}{2}R + C_{vib}$

where C_{vib} is the heat capacity resulting from the atomic vibration in the gaseous GeO molecule and is equal to the Einstein heat capacity function $E(\theta/T)$.

Hence

$$\Delta C_p = -0.67-6.67\times10^{-3}T - 0.20\times10^5T^{-2}+E(\theta/T)$$

On integration we botain

$$\Delta H = \int_0^T \Delta C_p dT = \Delta H_0 - 0.67T - 3.335\times10^{-3}T^2 + 0.20\times10^5T^{-1} + \int_0^T E\left(\frac{\theta}{T}\right)dT \qquad (5)$$

where ΔH_0 is the constant of integration. Then the free energy equation is obtained as

$$\Delta G = \Delta H_0 + 0.67T\ln T + 3.335\times10^{-3}T^2 + 0.10\times10^5T^{-1} - T\int_0^T \frac{dT}{T^2}\int_0^T E\left(\frac{\theta}{T}\right)dT + JT \qquad (6)$$

3) K. K. Kelley, *Bur. Mines Bulletin*, No. 476 (1949).

where J is another constant of integration.

These two constants were evaluated in the usual manner. With the relation

$$\Delta G = -RT \ln p_{atm} = -4.575T \log p_{atm}$$

equation (6) is changed to

$$\sum = -4.575 \log p - 0.67 \times 2.303 \log T - 3.335 \times 10^{-3} T - 1 \times 10^{4} T^{-2} + \int_0^T \frac{dT}{T^2} \int_0^T E\left(\frac{\theta}{T}\right) dT = \frac{\Delta H_0}{T} + J$$

$E(\theta/T)$ was evaluated for each temperature from the table[4] as $E(\theta/T) = 1/3\, C_v$ with the characteristic temperature $\theta = 1{,}411$ which in turn was calculated from Barrow's spectroscopic data[5]. Integration and double integration of $E(\theta/T)$-function were carried out graphically. By means of the method of least squares using twelve $\sum$ functions, ΔH_0 and I were obtained:

$$\Delta H_0 = 122{,}122 \pm 285$$

and

$$J = -73.583$$

With these values, the heat of reaction, the free energy and the entropy change at 298°K were calculated from equations (5) and (6) as follows:

$$\Delta H_{298} = 121.7 \pm 0.3 \text{ kcal./mol.}$$

$$\Delta G_{298} = 101.7 \text{ kcal./mol.}$$

$$\Delta S_{298} = 67.3 \text{ cal./mol./deg.}$$

The heat of reaction can also be obtained by combining the thermal data from the literature (Table II).

TABLE II

Reaction	ΔH_{298}, kcal.
$GeO_2(s) = Ge(s) + O_2(g)$	128.3 ± 1.0[6]
$Ge(s) = Ge(g)$	91.5 ± 3.0[7]
$1/2\ O_2(g) = O(g)$	59.2
$Ge(g) + O(g) = GeO(g)$	-159.0[8]
$GeO_2(s) = GeO(g) + 1/2\ O_2(g)$	120.0 ± 4 kcal

It must be remembered that the heat of formation of solid germanium dioxide, 128.3 kcal., is referred to its amorphous state. The heat of transition GeO_2 (amorph.) $= GeO_2$ (cryst.) is unknown, but considering the case of silicon dioxide, it may not be bold to take it as high as 2 kcal. Adding this, the heat of reaction is obtained as 122.0 kcal., which is in good accord with our experimental value of 121.7 kcal.

The heat of dissociation of gaseous germanium monoxide cited above, 159 kcal., was calculated by Herzberg from the spectroscopic evidence by Birge-Sponer linear extrapolation. Though the applicability of the linear extrapolation to all oxides of group-4 elements was discussed by Brewer[1,9], our experimental result also suggests that Herzberg's value is preferable to Gaydon's, 127 ± 27 kcal.

From $\Delta S_{298} = 67.3$ e. u., together with the entropy values from the literature[10] $S_{298} = 49.01 \pm 0.1$ e. u. for oxygen and $S_{298} = 53.55 \pm 0.1$ e. u. for gaseous germanium monoxide, the entropy of solid germanium dioxde is obtained as $S_{298} = 10.8 \pm 1.0$ e. u. This is in fair agreement with the estimated value 11.5 which was evaluated by Bues and von Wartenberg, by interpolation from normal entropies of dioxides of silicon and tin[11].

Summary

The apparent vapor pressure of solid germanium dioxide, or the dissociation pressure for the reaction $GeO_2(c) = GeO(g) + 1/2\, O_2(g)$, was measured over the temperature range 1,040–1,100°C by the Knudsen's effusion method; the results are expressed by equation (4).

From the free energy equation, the heat of reaction, the free energy and the entropy change of the reaction were calculated as $\Delta H_{298} = 121.7 \pm 0.3$kcal./mol., $\Delta G_{298} = 101.7$ kcal./mol. and $\Delta S_{298} = 67.3$ e. u., respectively. The heat of reaction is in good agreement with the value calculated from accessible thermal data. The normal entropy of solid germanium dioxide was calculated as $S_{298} = 10.8 \pm 1.0$ e. u., which is in agreement with the computed value obtained by Bues and von Wartenberg.

We are indebted to Professor Saburo Yanagizawa of Keio University, for his interest in this research to furnish us with the sample of pure germanium dioxide.

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4) Landolt-Börnstein, Tabellen, Erg. I., p. 702.
5) R. F. Barrow, *Proc. Phys. Soc.*, **56**, 204 (1944).
6) J. P. Coughlin, *Bur. Mines Bulletin*, No. 542 (1954).
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9) L. Brewer and D. F. Mastick, *J. Chem. Phys.*, **19**, 834 (1951).
10) K. K. Kelley, *Bur. Mines Bulletin*, No. 477 (1951).
11) W. Bues and H. von Wartenberg, *Z. anorg. allg. Chem.*, **266**, 281 (1951).

Rapid Determination of Inorganic Sulfur in Various Forms, Particularly in Sulfide Ores, by the Tin(II)-Strong Phosphoric Acid Reduction Method

By Toshiyasu KIBA, Ikuko AKAZA and Nobuyuki SUGISHITA

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Previously it was shown that both soluble and insoluble sulfates could be easily reduced to hydrogen sulfide by heating with tin(II)-strong phosphoric acid, a new powerful and stable reducing agent first described by the authors[1]. The discovery of this reagent led the authors to the development of a new rapid and simple method for the determination of sulfate sulfur, in which hydrogen sulfide evolved by the reaction was absorved by zinc acetate solution and titrated iodimetrically or estimated colorimetrically as usual. Both the volumetric procedure for semimicro scale and the colorimetric method for micro quantities of sulfate were already established by the authors and have been widely accepted in this country for investigative and industrial purposes[1,2]. Extension of this method to rapid determination of organic sulfur was also accomplished in this laboratory[3]. The organic sulfur was oxidized to sulfate by means of chromium(VI)-strong phosphoric acid and then estimated in the same way as inorganic sulfates, the volumetric or the colorimetric method being used in the final stage according to the sulfur content of the sample[4].

The present paper describes a later investigation in which the above method was extended to the determination of inorganic sulfur in other forms, e. g., thiosulfates, sulfites, thiocyanates and sulfides, in particular to rapid estimation of sulfur in sulfide ores such as pyrite, galena and zinc blende. The rapidity and the simplicity of the original method are retained in this procedure, in which all the sulfur in the samples is converted into hydrogen sulfide and determined iodimetrically.

Experimental

Apparatus:—The apparatus used in this study is shown in Fig. 1. It is composed of two parts,

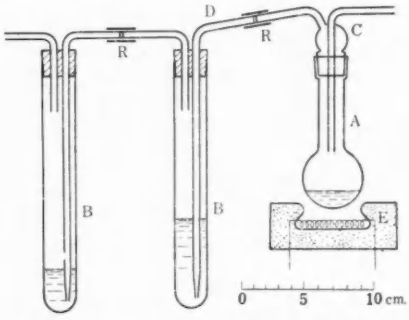

Fig. 1. Apparatus for reduction and absorption.
A: Reaction vessel, a round bottom flask
B: Absorbing vessels
C: Glass cap fitted to the reaction vessel
D: Gas-delivery tube
R: Short pieces of rubber tubing
E: Electric heater

a reaction vessel (A) and an absorption part (B). The reaction vessel (A) is a round bottomed flask of hard glass having a glass cap fitted to its top and provided with inlet and outlet tubes. The absorption part (B) is composed of two test-tubes 3 cm. in diameter. A gas delivery tube (D) is connected with a piece of rubber tubing to the outlet arm of the reaction vessel (A) and inserted into the absorbing solution. A Kipp's apparatus is employed for the production of carbon dioxide, which is purified by six gas-washing bottles (not shown Fig. 1.) and introduced into the reaction vessel through the inlet arm. The solutions used for the purification of carbon dioxide are described below.

Reagents:—*Storong Phosphoric Acid.*—Four hundred grams of commercial ortho phosphoric acid of extra pure grade (d=1.7) is placed in a 300-ml. flask or a 350 ml. conical beaker, and dehydrated by heating on a hot plate until a thermometer dipped in the liquid indicates 300°C. If the heating takes too long a time the liquid will become extremely turbid and viscous, and useless for the purpose in view. During the heating, the water vapor and the mist of phosphoric acid evolved should be rapidly removed from the neighborhood of the liquid surface through a glass tube, an end of which is hung down near the liquid surface, the other end being connected to a suction pump. It saves much time in heating.

1) T. Kiba et al., This Bulletin **28**, 641 (1955).
2) T. Kiba and I. Kishi, (present surname Akaza), ibid., **30**, 44 (1957).
3) S. Ohashi, ibid., **28**, 645 (1955).
4) T. Kiba, I. Akaza and S. Taki, ibid., **30**, 482 (1957).

Tin(II)-Strong Phosphoric Acid.—Fifty grams of tin(II)-chloride dihydrate of extra pure grade is placed in a 300-ml. conical beaker and on it, is poured 250 g. of the strong phosphoric acid prepared above. The content is heated within about one hour to 300°C after the manner of the preparation of the strong phosphoric acid. After cooling the tin(II)-strong phosphoric acid thus obtained, a very viscous and occasionally turbid liquid, may be stored in a closed vessel.

Zinc Acetate Solution, 4% or 8%.—Forty or 80 g. of zinc acetate dihydrate of pure grade is dissolved in 1 l. of distilled water. If necessary, two or three drops of glacial acetic acid are added to clarify the solution, but the pH of the solution should not fall below 5. A solution of suitable concentration is used depending upon the amount of sulfur to be analyzed.

Iodine, 0.02 N or 0.05 N.—These are standardized accurately by the ordinary procedure. Either of them is used depending on the amount of sulfur to be analyzed.

Sodium Thiosulfate, 0.02 N or 0.05 N.—These are standardized accurately by the ordinary procedure.

Carbon Dioxide.—Carbon dioxide produced in the Kipp's apparatus is purified by passing it through five gas-washing bottles containing, respectively, water, 1% potassium permanganate in 10% sodium carbonate solution, 2% vanadium(II) sulfate in 6N sulfuric acid solution[3], 5% barium chroride, and a zinc acetate absorbing solution. A sixth empty bottle is placed to intercept the droplets coming from the washing liquids.

Barium Hydroxide Solution.—Barium hydroxide dihydrate crystals of pure grade are saturated in distilled water and stored away from atomospheric carbon dioxide.

Bromine Water.—Bromine of pure grade is saturated in distilled water.

Various Samples Containing Sulfur.—Pure grade reagents of sodium thiosulfate, barium thiosulfate, sodium sulfite, potassium thiocyanate, and sodium sulfide were employed for the evaluation of the method. The salts were dissolved in water to prepare solutions of known concentration. The sulfur content of a definite volume of each solution was estimated by the conventional gravimetric or volumetric method. The sulfide ores used were natural pyrite, zinc blende and galena, from Ogoya Mine near Kanazawa city, and their sulfur contents were estimated by the gravimetric analysis according to the procedure described in ordinary text-books.

Procedure:—*Procedure A.*—Take an accurately measured sample—pipet a solution; weigh a solid—containing less than 20 mg. of sulfur and put it into the reaction vessel (A, Fig. 1). Add 0.5 ml. of the barium hydroxide solution, and evaporate the content to dryness at the bottom of the vessel. Pour 10 ml. of tin(II)-strong phosphoric acid over the dried residue with a pipet. Put 40 ml. of the zinc acetate solution into the first absorbing vessel and 20 ml. of the same solution into the second. Connect all the apparatus as shown in Fig. 1. Pass carbon dioxide very rapidly through the apparatus for five minutes to expel the air from the apparatus. Then reduce the flow of the gas to a rate of one bubble per second. Heat the bottom of the reaction vessel on a small electric heater which is regulated by a variable transformer, until white fumes of the strong phosphoric acid appear. It is unnecessary to keep the reaction temperature constant, but it should be high enough to ensure that the content of the vessel exhibits a white turbidity near the end of the reaction. Stop the heating and continue the passage of carbon dioxide for five minutes.

Disconnect the absorbing vessels from the gas-outlet arm of the reaction vessel and quickly pour into them 20 ml. of 0.02 N iodine solution—the bulk into the first vessel and only a bit into the second. A part of the zinc sulfide precipitate may cling to the glass tubes; it is allowed to remain in the absorbing vessels. Swirl the vessel to mix the content thoroughly, transfer it to a beaker and back titrate the excess of the iodine with 0.02 N sodium thiosulfate solution using starch as indicator.

The whole determination can be carried out in thirty or forty minutes. A blank test should be run without a sample. One ml. of 0.02 N iodine corresponds to 0.3206 mg. of sulfur.

Procedure B.—Take the sample into the reaction vessel. Add two or three drops of the bromine water to oxidize the sulfur to sulfate form and then fix it as barium sulfate by adding 0.5 ml. of the barium hydroxide solution. Evaporate the content on a water-bath, and carry out the reduction of the sulfate according to Procedure A as shown above.

Thiosulfates, thiocyanates and sulfites should be treated by procedure B before the reduction with tin(II)-strong phosphoric acid.

Results and Discussion

Sulfide Ores.—Zinc blende, galena and pyrite were analyzed using procedure A. In Table I the results obtained by the gravimetric method are tabulated together. As all the sulfur in these sulfide ores could be generated as hydrogen sulfide by the simple treatment with the tin(II)-strong phosphoric acid, all troublesome treatments such as dissolving of the ores in acids and evaporation of the solution were dispensed with, in the course of the analysis. The determination of the sulfur could be accomplished very quickly—within twenty or thirty minutes. Because of the relatively small amount of the sample employed in this method care should be taken to grind the ore into fine powder and to mix it thoroughly, so that the sample may correctly represent the whole. The standard deviation (σ) and the average deviation (δ) of the results obtained by this method were comparable to those

TABLE I

DETERMINATION OF SULFUR IN SULFIDE ORES

A: Zinc Blende

Sample Taken[a] mg.	S Found mg.	S Content[b] %
37.46	11.84	31.60
37.47	11.63	31.03
40.33	12.64	31.34
42.65	13.18	30.90
47.69	15.03	31.49

($\sigma = 0.246\%$, $\delta = 0.298\%$)

a) The sample ore was ground into 200-mesh and weighed by a micro-balance.

b) The mean value of sulfur content obtained by gravimetric method was 30.12%.

B: Galena

By Procedure A			By Gravimetric Method	
Sample Taken[c] mg.	S Found mg.	S %	Sample Taken g.	S Found %
73.52	9.38	12.75	0.2626	12.32
76.56	9.85	12.86	0.2900	12.79
82.53	10.48	12.69	0.2820	13.02
83.92	10.72	12.77	0.2685	12.09
96.19	12.13	12.61	0.3477	12.47
($\sigma = 0.068\%$, $\delta = 0.093\%$)			($\sigma = 0.294\%$, $\delta = 0.370\%$)	

c) The sample ore was ground into 200-mesh and weighed by a micro-balance.

C: Pyrite

By Procedure A			By Gravimetric Method (According to Lunge)	
Sample Taken[d] mg.	S Found mg.	S %	Sample Taken g.	S Found %
16.50	8.61	52.18	0.0838	51.88
17.82	9.12	51.17	0.1107	51.04
26.63	13.90	52.19	0.1490	52.10
27.43	14.13	51.51	0.1252	52.14
28.51	14.61	51.24	0.1402	50.98
($\sigma = 0.422\%$, $\delta = 0.497\%$)			($\sigma = 0.488\%$, $\delta = 0.572\%$)	

d) The sample ore was ground into 200-mesh and weighed by a micro-balance.

by the gravimetric analysis. In both cases the ores were ground to about 200 meshes (per inch). Slightly higher values of sulfur content were obtained by this than by the gravimetric method. Probably because no sulfur in the sample was lost during the couse of this method, while with the gravimetric method, a small amount of the sulfur might escape as sulfur dioxide or hydrogen sulfide during the process of dissolution in acid and evaporation of the solution. The results obtained by this method may thus be regarded as more reliable.

Sulfide.—An aliquot of freshly prepared sodium sulfide solution was employed for the analysis, the sulfur content having been estimated by iodimetry. Two methods were employed here. In one of them procedure B was employed, and in the other a few drops of the zinc acetate solution were added to the sample and the sulfur was analysed by procedure A. The results obtained by the above two methods are shown in Table II, from which it is revealed that whether the oxidation of sulfide ion takes place or not, all of the sulfur in the sample is ultimately converted in to hydrogen sulfide by this reduction method.

TABLE II

DETERMINATION OF SULFUR IN SODIUM SULFIDE

S Contained in an Aliquot[a] mg.	S Found By This Method mg.	Note
2.99	2.98	Zinc acetate was added.
〃	2.99	〃
〃	2.94	〃
〃	2.93	〃
〃	2.99	By procedure B.
〃	3.01	〃
〃	3.00	〃

a) The amount of the sulfide ion in an aliquot was determined by iodimetry.

TABLE III

DETERMINATION OF SULFUR IN THIOSULFATES

A: Barium thiosulfate $BaS_2O_3 \cdot H_2O$ (Purity of the sample: 99.95%) S content: 23.96%, analysis was carried out by procedure A.

Sample Taken mg. (A)	S Content (Calcd.) ($A \times 0.2396$) mg.	S Found mg.	S Found %
10.06	2.41	2.40	23.85
22.63	5.42	4.88	21.56
24.35	5.83	5.42	22.25
10.06	2.41	2.41	23.95
〃	〃	2.41	23.95
〃	〃	2.39	23.75
〃	〃	2.42	24.05
〃	〃	2.40	23.85
17.24	4.13	4.15	24.07
17.25	4.13	4.16	24.11
19.46	4.66	4.65	23.89

B: Sodium Thiosulfate $Na_2S_2O_3 \cdot 5H_2O$ (S content: 25.84%)

Sample Taken mg. (A)	S Content (Calcd.) ($A \times 0.2584$) mg.	S Found mg.	S Found %
17.16	4.43	4.44	25.87
〃	〃	4.42	25.75
〃	〃	4.43	25.81
8.58	2.21	2.22	25.87
〃	〃	2.22	25.87

Thiosulfate.—Sodium thiosulfate solution and barium thiosulfate solid were analyzed by procedure B. The thiosulfate ion could not be reduced quantitatively to hydrogen sulfide merely by heating with the tin(II)-stong phosphoric acid. It had to be oxidized to sulfate before the reduction. Assay of the sulfur in the sample was carried out by iodimetry. The results obtained are shown in Table III.

Sulfite.—An aliquot of sodium sulfite solution was employed for the analysis. The solution usually contains both sulfite and sulfate ions, for the former is easily oxidized to the latter during the preparation and storage of the solution; the content of the total sulfur, however, remains unaffected. The analysis was carried out according to procedure A and B, but the results coincide closely with each other as shown in Table IV.

TABLE IV

DETERMINATION OF SULFUR IN SODIUM SULFITE

Sample Taken mg. (A)	S Content (Calcd.) ($A\times0.2059$) mg.	S Found mg.	S Found %	Procedure
20.14	4.15	4.14	20.55	A.
〃	〃	4.16	20.65	〃
〃	〃	4.14	20.55	〃
〃	〃	4.21	20.90	B.
〃	〃	4.13	20.50	〃
〃	〃	4.21	20.90	〃

a) Purity of the reagent as a solid: Na_2SO_3 60.225%

b) Total sulfur content of an aliquot taken was gravimetrically determined as barium sulfate; sulfur content: 20.59%.

TABLE V

DETERMINATION OF SULFUR IN POTASSIUM THIOCYANATE

Sample Taken ml. (A)	S Content (Calcd.) ($A\times3.84$ mg.)	S Found mg.	S Found %	Procedure
2.0	7.68	2.56	11.04	A.
5.0	19.20	4.19	0.72	〃
2.0	7.68	7.64	32.83	B.
〃	〃	7.65	32.87	〃
〃	〃	7.74	33.26	〃
〃	〃	7.69	33.04	〃
3.0	11.52	11.38	32.60	〃
〃	〃	11.58	33.18	〃
〃	〃	11.50	32.95	〃

a) Sulfur content of potassium thiocyanate was calculated from the results obtained by argentiometry. One milliliter of the solution used here contained 3.84 mg. of sulfur.

Thiocyanate.—A solution of potassium thiocyanate was used for the analysis. As sulfur in a thiocyanate can not be reduced to hydrogen sulfide merely by treatment with the tin(II)-strong phosphoric acid, procedure B was adopted for this compound. Results obtained by procedures A and B are listed together in Table V.

The determination of inorganic sulfur in various forms can be done by procedure A or by procedure B within thirty minutes. With reference to rapidity of analysis this method is greatly superior to all the conventional ones. In accuracy this method is comparable to gravimetric methods, but the amount of sample to be taken for analysis is far smaller than that needed for other routine methods, as shown in Table I in the case of sulfide ores.

Summary

The procedure for the determination of sulfate sulfur using a new reagent, tin (II)-strong phosphoric acid, previously devised by the authors, has been extended to the determination of sulfur in other forms of inorganic compounds, e.g., sulfide ores (zinc blende, galena and pyrite), sulfides, thiosulfates, sulfites and thiocyanates. Two procedures of the analysis have been proposed by the authors: one, suitable for sulfide ores, sulfides, and sulfites, in which samples are treated directly with tin(II)-strong phosphoric acid to reduce the sulfur to hydrogen sulfide, and the other, suitable for sulfites, thiosulfates, and thiocyanates, in which samples are previously treated with bromine water to oxidize the sulfur to sulfate, and the sulfate is reduced to hydrogen sulfide. The hydrogen sulfide evolved from the sample is absorbed in zinc acetate solution and titrated iodimetrically as usual. The rapidity and the simplicity of the original method are still retained in this case, by which less than 30 milligrams of sulfur in the sample can be estimated within thirty minutes with good reproducibility.

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Studies on Serine Peptides. III. Synthesis of L-Seryl-L-Histidine, L-Seryl-L-Histidyl-L-Leucine and Carbobenzyloxy-Glycyl-O-Benzyl-DL-Serine Ethyl Ester

By Kenji OKAWA

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Seryl-histidyl peptide was found in insulin by Sanger, who suggested that the peptide plays an important role in the biological action[1]. It was reported that the same peptide groups might also be contained in ribonuclease[2] and chymotrypsin[3].

With regard to synthetic studies of seryl peptides, the author reported in the previous paper[4] that the *O*-Bz*-L-serine was a useful starting material for the synthesis of seryl peptides, and *N*-Cbzo**-*O*-Bz-L-seryl-L-histidine methyl ester was synthesized in a good yield. Although the synthetic studies on histidyl peptides have been reported by Holley and Fischer[5,6] only a few simple peptides were obtained by the azide method in an unsatisfactory yield. Fischer and Whetston reported that Cbzo-glycyl-L-histidine azide was coupled with L-serine methyl ester or L-tyrosin methyl ester in the yields of 25 and 43% of theory, respectively.

In the present work, *N*-Cbzo-*O*-Bz-L-seryl-L-histidine was coupled with leucine methyl ester in dioxane by the use of Sheehan's dicyclohexylcarbodiimide[7] to give *N*-Cbzo-*O*-Bz-L-seryl-L-histidyl-L-leucine methyl ester in 68% yield. The free peptides, L-seryl-L-histidine and L-seryl-L-histidyl-L-leucine, were prepared from their Cbzo-*O*-benzyl derivatives by treatment with hydrogen bromide in dioxane. The esterification of *O*-Bz-L- and DL-serine was carried out in the ethanol-dry hydrogen chloride solution at low temperature. *N*-Cbzo-glycyl-*O*-Bz-DL-serine ethyl ester was obtained in 88% yield by Sheehan's method.

Acknowledgement: Low yields in the synthesis of histidyl peptides by means of azide method are possibly caused by the condensation of imidazole and acyl azide group.

This difficulty was overcome by the use of dicyclohexylcarbodiimide method. *N*(α)-Cbzo-histidine still has a zwitter ion character owing to the presence of imidazole ring, nevertheless the amino group is carbobenzoxylated, and it is difficultly soluble in usual organic solvents. On the contrary, *N*-Cbzo-*O*-Bz-L-seryl-L-histidine is not soluble in hot water but soluble in dioxane and chloroform. This fact favors the homogeneous reaction giving high yields of the reaction product.

The author tried to remove the protecting groups of the *N*-Cbzo-*O*-Bz-L-serine by using dry hydrogen bromide in glacial acetic acid according to the Ben-Ishai's method[8], but hereby *O*-acetyl-serine was obtained in place of free L-serine. In dioxane, the elimination of protective groups from peptide derivatives proceeded smoothly and free peptides were obtained in desirable yields. It is interesting that the benzyl residue in *O*-Bz-serine is not attacked in the esterification procedure but is readily cleft by the use of hydrogen bromide in dioxane. The results provide further evidences that the free hydroxy group of serine must be protected by a suitable group to obtain good results in peptide synthesis.

Experimental

N-Cbzo-O-Bz-L-seryl-L-histidine. —*N*-Cbzo-*O*-Bz-L-seryl-L-histidine methyl ester[9] was prepared from *N*-Cbzo-*O*-B_2-L-serine and L-histidine methyl ester by Sheehan's method. Into the solution of 25 g. of the carbobenzoxy-peptide ester in 52 cc. of dioxane was added 52 cc. of aqueous solution of

* Bz-; Benzyl-

** Cbzo-; Carbobenzyloxy-

1) *Symposium on Peptide Chemistry*, 1955 (London), p. 24.

2) C. H. W. Hirs, *Fed. Proc.*, **16**, 196 (1957), R. R. Redfield and C. B. Anfinsen, *J. Biol. Chem.*, **221**, 385 (1956). C. H. W. Hirs, W. H. Stein and S. Moore, ibid., **221**, 151 (1956).

3) J. R. Whitaker and B. J. Jandorf, *J. Biol. Chem.*, **223**, 751 (1956).

4) K. Okawa, This Bulletin, **29**, 486 (1956).

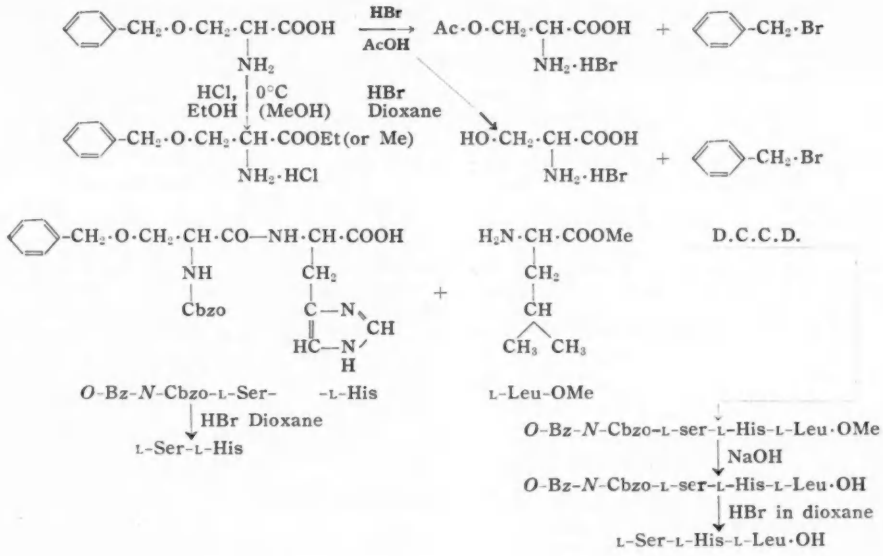
5) R. W. Holley and E. Sondheimer, *J. Am. Chem. Soc.*, **76**, 1326 (1954).

6) R. F. Fischer and R. R. Whetstone, ibid., **76**, 5076 (1954).

7) J. C. Sheehan and G. P. Hess, ibid., **77**, 1067 (1955).

8) Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

9) K. Okawa, This Bulletin, **29**, 489 (1956).



1 N-sodium hydroxide portion-wise in 20 minutes under stirring. After kept standing for 30 minutes, the reaction mixture was brought to pH 2.0 with hydrochloric acid, and washed with ethylacetate. The aqueous layer was brought to pH 4.4, and 18.0 g. of Cbzo-peptide was precipitated, m.p. 156°C. Recrystallization from methanol-ether gave 16.0 g. of pure material, m.p. 164°C. in 66.8 % yield. $[\alpha]_D^{13}=+20.7$ (10 mg/cc. AcOH)

Anal. Found: C, 60.40; H, 5.39; N, 12.04. Calcd. for $C_{24}H_{26}O_6N_4$: C, 61.80; H, 5.58 N, 12.02 %.

***N*-Cbzo-*O*-Bz-L-seryl-L-histidyl-L-leucine Methyl Ester.**—Two grams of *N*-Cbzo-*O*-Bz-L-seryl-L-histidine in 80 cc. of dioxane was coupled with 1 g. of dicyclohexylcarbodiimide in 20 cc. of tetrahydrofurane and L-leucine methyl ester in 10 cc. of tetrahydrofurane (prepared from 1.0 g. of its hydrochloride by the use of ammonia-chloroform solution[10]). After the solution was left at room temperature overnight, 0.3 cc. of acetic acid was added to remove the unchanged dicyclohexylcarbodiimide, and the urea derivative produced was filtered off. The filtrate was concentrated in vacuo, the residue dissolved in ethylacetate, the solution washed with dilute acid, alkali and water and then dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residual precipitate was collected and washed with petroleum ether. The yield was about 2.0 g. Recrystallization from ethylacetate-petroleum ether gave 1.5 g. of carbobenzoxypeptide ester. From the mother liquor, 0.1 g. of the crystals were obtained. The total yield was 1.6 g. (64 %), m.p. 152-153°C. $[\alpha]_D^{13}=-20.14$ (10 mg./cc. EtOH)

Anal. Found: C, 62.60; H, 6.60; N, 11.61. Calcd. for $C_{31}H_{39}O_7N_5$: C, 62.73; H, 6.57; N, 11.79 %.

***N*-Cbzo-*O*-Bz-L-seryl-L-histidyl-L-leucine.**—By the usual method, 1.2 g. of the peptide ester was dissolved in 2cc. of dioxane and 2 cc. of an aqueous solution of 1 N sodium hydroxide was added portion-wise into dioxane solution and the reaction mixture was allowed to stand for 50 minutes at room temperature. When the hydrolysis was over, 1 cc. of 2 N hydrochloric acid was added into the solution, and the fine precipitate produced was filtered (1.1 g.). Recrystallization from methanol gave 0.7 g. of the carbobenzoxypeptide in 68.7 % yield, m.p. 172-173°C.

Anal. Found: C, 61.00; H, 6.17; N, 11.99. Calcd. for $C_{30}H_{37}O_7N_5$: C, 62.17; H, 6.38; N, 12.08 %.

***O*-Ac-L-serine.**—Dry hydrogen bromide was passed into the solution of *N*-Cbzo-*O*-B_2-L-serine (1.1 g.) in 7 cc. of acetic acid for twenty minutes. After the solution was left at room temperature for 30 minutes, 70 cc. of absolute ether was added into the reaction mixture. The white precipitate produced was washed twice with dry ether. The products (0.65 g., 94.2 %) were dried in a vaccum desiccator over sodium hydroxide.

By treating this product with triethylamine in absolute ethanol, *O*-Ac-L-serine was obtained.

Anal. Found: C, 40.35; H, 5.87; N, 9.56. Calcd. for $C_5H_9O_4N$: C, 40.81; H, 6.09; N, 9.52 %.

Dioxane was used in place of acetic acid in the above procedure. The crystals of L-serine monohydrogen bromide could be obtained in 90 % yield.

L-Seryl-L-histidine Mono Hydrogen bromide.—Dry hydrogen bromide was passed into the suspension of 1 g. of *N*-Cbzo-*O*-Bz-L-seryl-L-histidine in 7 g. of dioxane at room temperature

10) G. Hillmann, *Z. Naturforsch*, **1**, 682 (1946).

until the concentration of hydrogen bromide reached 25% and then the mixture was allowed to stand for an hour. In this reaction period, the carbobenzoxy-peptide was dissolved perfectly into the dioxane solution. To the reaction mixture was added dry ether and the precipitate was collected and washed with ether three times. The precipitate was likely to be slightly hygroscopic. The yield was about 1.2 g. The precipitate was dissolved in 5 cc. of ethanol and dry pyridine was added until the solution became slightly turbid (pH 6.5). After a few minutes, the colloidal precipitate was removed and the filtrate was allowed to stand for an hour in an ice-box. Fine powdery crystals (300 mg.) were obtained, m.p. 205–207°C with decomposition. (in 57% yield) $[\alpha]_D^{13} = +26.43$ (30 mg./cc. water)

Anal. Found: C, 33.17; H, 4.48; N, 16.88. Calcd. for $C_9H_{15}O_4N_4Br$: C, 33.43; H, 4.33; N, 17.33%.

L-Seryl-L-histidyl-L-leucine. — *N*-Cbzo-*O*-Bz-L-seryl-L-histidyl-L-leucine (0.5 g.) was dissolved in 5 cc. of dioxane and dry hydrogen bromide was passed to produce about 25% solution. After standing for an hour at room temperature, dihydrobromide of free peptide (0.4 g.) was precipitated on addition of dry ether. The precipitated dihydrobromide was dissolved in 3 cc. of absolute ethanol, followed by addition of 0.2 cc. of triethylamine and the mixture was allowed to stand overnight in an ice-box. The free peptide (300 mg., m. p. 215–216°C with decomposition) was obtained. Recrystallization from ethanol containing a small amount of water gave 200 mg. of the pure peptide in 70% yield, m. p. 230°C with decomposition. $[\alpha]_D^{13} = -25.3$ (10 mg./cc. water)

Anal. Found: C, 49.03; H, 6.90; N, 18.84. Calcd. for $C_{15}H_{25}O_5N_5 \cdot H_2O$: C, 48.22; H, 7.20; N, 18.80%.

***O*-Bz-DL-serine Ethylester Hydrochloride.**— Ten grams of *O*-Bz-DL-serine was suspended in 100 cc. of absolute ethanol and the solution was saturated with dry hydrogen chloride at 0°C. After kept standing overnight at 5°C the mixture was evaporated in vacuo and this procedure was repeated. The residual crystals were recrystallized twice from ethanol-ether. The yield (11 g.) was about 80%, m.p. 118°C.

Anal. Found: C, 54.90; H, 6.79; N, 5.59. Calcd. for $C_{12}H_{15}O_3NCl$: C, 55.49; H, 6.98; N, 5.40%.

Cbzo-glycyl-*O*-Bz-DL-serine Ethylester. — *O*-Bz-DL-serine ethylester was prepared from 5.2 g. of its hydrochloride by the use of ammonia-chloroform solution. This free ester in 30 cc. of chloroform was coupled with 2.9 g. of Cbzo-glycine in 20 cc. of chloroform and 3.1 g. of dicyclohexylcarbodiimide in 10 cc. of chloroform. The reaction mixture was allowed to stand overnight, and shaken with 5 cc. of 0.5 N hydrochloric acid to remove the unchanged dicyclohexylcarbodiimide. After filtration of the dicyclohexyl urea produced, the filtrate was washed with dilute acid, alkali and water, and then dried over anhydrous sodium sulfate. The solvent was removed in vacuo. By adding petroleum ether to the residual, 5.1 g. of Cbzo-glycyl-*O*-Bz-DL-serine ester was obtained in 88% yield, m.p. 80–83°C. Recrystallization from ethylacetate-petroleum ether gave the pure material, m. p. 84°C.

Anal. Found: C, 63.81; H, 6.58; N, 6.84. Calcd. for $C_{22}H_{26}O_6N_2$: C, 63.74; H, 6.32; N, 6.76%.

Cbzo-glycyl-*O*-Bz-DL-serine Hydrazide. — Into the solution of 2.1 g. of Cbzo-glycyl-*O*-Bz-DL-serine ethyl ester in 40 cc. of ethanol was added 0.3 g. of hydrazine hydrate and the mixture was allowed to stand overnight at room temperature. The crystals produced were collected and washed with ethanol. Recrystallization from hot ethanol gave 1.8 g. of hydrazide in 90% of theory, m.p. 188–189°C.

Anal. Found: C, 60.22; H, 5.50; N, 14.08. Calcd. for $C_{20}H_{24}O_5N_4$: C, 59.99; H, 6.04; N, 13.99%.

***O*-Bz-L-serine Methylester Hydrochloride.** —From 5.7 g. of *O*-Bz-L-serine, 7.2 g. of its methyl ester was obtained by the same method as in the case of O-Bz-L-serine ethyl ester. Recrystallization from methanol-ether gave 6.5 g. of pure crystals in 90% yield, m.p. 169°C. $[\alpha]_D^{13} = -2.7$ (30 mg./cc. water)

Anal. Found: C, 53.76; H, 6.20; N, 5.52. Calcd. for $C_{11}H_{16}O_3NCl$: C, 53.77; H, 6.54; N, 5.70%.

Summary

L-Seryl-L-histidine and L-seryl-L-histidyl-L-leucine have been synthesized in good yields by Sheehan's dicyclohexylcarbodiimide method. In the removal of protecting groups of *O*-Bz-serine derivatives by Ben-Ishai's method, dioxane must be used as the solvent in place of glacial acetic acid. *O*-Bz-L- and DL-serine ester was synthesized the mild esterification condition.

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Chemical Constitution of Renieratene

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(Received August 7, 1957)

Renieratene is the main consistuent of carotenoids of a sponge "*Reniera japonica*"[1,2]. The most conspicuous characteristic of the pigment is that it has the most unsaturated composition of all the carotenoids hitherto reported. The light absorption maxima of the pigment, however, are located at nearly the same position as those of γ-carotene, which has only twelve double bonds in the molecule.

It has been reported in the previous paper[2] that renieratene has the molecular formula $C_{40}H_{48}$ or $C_{40}H_{50}$ (the former is more probable). Further confirmation of its molecular weight has been given by means of isothermal distillation. On catalytic hydrogenation, renieratene absorbs 15 moles of hydrogen. It is therefore considered to be a cyclic compound with one or two rings.

The quasi-equilibrium mixture of *cis-trans* isomerization of the pigment with iodine, shows the absorption maxima at a shorter wavelength region than those of the original solution, and a marked *cis*-peak also appears (Fig. 1). It can therefore, be concluded that renieratene is not a *cis*-isomer of a carotenoid with the absorption maxima of longer wavelength but has an all-*trans* form as to the configuration of conjugated double bonds. High melting point and low solubility of the pigment also support the conclusion.

The infrared spectrum (Fig. II) indicates the absence of vinyl group, which is further supported by the fact that renieratene is a rather stable carotenoid and has little tendency to polymerize. When a C_{40}—carotenoid with two 2,2,6-trimethylcyclohexyl-skeletons has no vinyl group, the maximum number of double bonds which are allowed to exist is fourteen, as in the case of anhydroeschscholtzxantin[3], I. If the carotenoid had throughout an open chain structure it might of course be possible to contain fifteen double

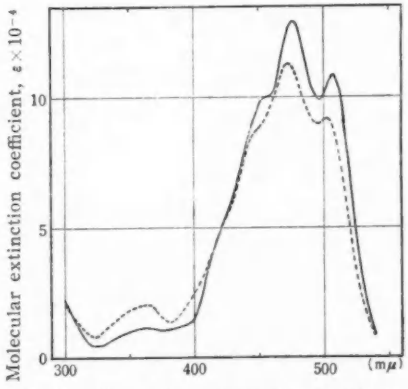


Fig. 1. Absorption curves of renieratene
—— fresh solution (benzene)
---- isomerized solution with iodine.

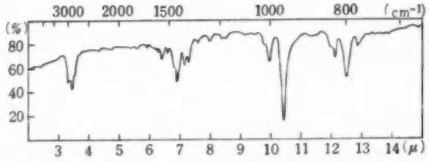


Fig. 2. Infra-red spectrum of renieratene.

bonds in the molecule. However even if it were so, all these double bonds would be in conjugation as in the case of dehydrolycopene[4], II; therefore the absorption maxima should be situated at much longer wavelength regions. These considerations lead to a presumption that a highly unsaturated group with a slight bathochromic effect must exist in the molecule of renieratene. There are two presumable cases which satisfy this requirement, i.e. the presence of triple bonds or the presence of benzene rings in the molecule of renieratene, although the former is less likely because of the reasons described below.

In the infra-red spectrum (Fig. II), no bands are observed which are characteristic of triple or allenic bond. On the other hand, a sharp absorption at 800 cm^{-1}, which seems to be characteristic of renieratene

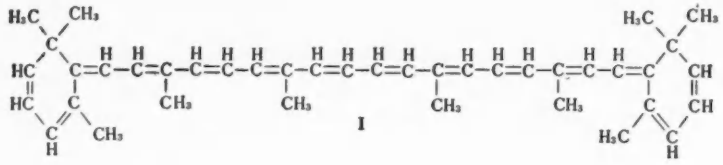
* Pigments of Marine Animals, IV.; III of this series; This Bulletin, **30**, 111 (1957).

1) T. Tsumaki, M. Yamaguchi and T. Tsumaki, *J. Chem. Soc. Japan, Pure Chem. Sec.*, **75**, 297 (1954).

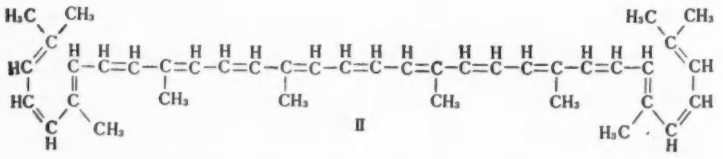
2) M. Yamaguchi, This Bulletin, **30**, 111 (1957).

3) P. Karrer and E. Leumann, ibid., **34**, 445 (1951).

4) P. Karrer and J. Rutschmann, *Helv. chim. Acta* **28**, 793 (1945).



I



II

because of the general lack of this absorption in common carotenoids, is considered to give an indication of the presence of the benzene ring with two or three adjacent free hydrogen atoms. However this interpretation is not decisive, for the absorption in 3030 cm^{-1}, 1500—1600 cm^{-1} and other regions which are useful for the identification of aromatic structures, are not distinctive, because of the simultaneous presence of the absorption due to the polyene structure in the same regions.

Renieratene gives only a very small amount of acetone (0.03—0.05 mole) on ozonolysis. It has been reported that one isopropylidene group gives 0.8—0.9 mole of acetone[5], one isopropyl group 0.3 mole[5] and an α- or a β-ionone group 0.15 mole[6]. Therefore it seems that renieratene has neither isopropylidene group nor ionone ring. This result seems to be in favor of the presumption of the presence of the benzene ring.

Renieratene has no provitamin A activity in the rat when applied in daily doses of 60 γ. This is in agreement with the results of the ozonolysis.

By the mild oxidation of reniertene in benzene-acetic acid soultion with chromic acid corresponding to two atoms of oxygen per mole of the pigment, followed by chromatographic separation, two pigments are obtained in small yield. One of them forms purple crystals of m. p. 199°, and the other forms brown crystals of m. p. 127°. Both the pigments have the formula of $C_{30}H_{36}O$ and are considered to be polyene aldehydes. Therefore these two pigments seem to be in an isomeric relation. The term *renieral* is tentatively proposed for the pigment of m. p. 199°, and *isorenieral* for the pigment of m. p. 127°. On catalytic hydrogenation, both the pigments absorb 12 moles of hydrogen. This

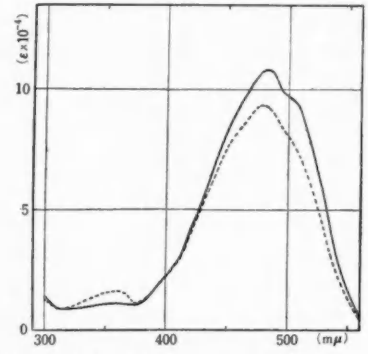


Fig. 3. Absorption curves of renieral.
—— fresh solution (benzene).
---- isomerized solution with iodine

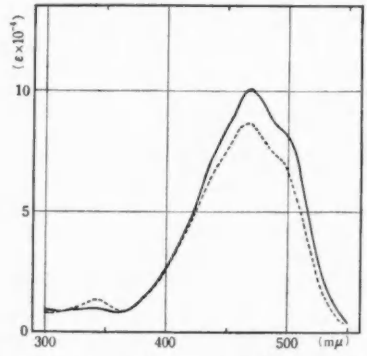


Fig. 4. Absorption curves of isorenieral.
—— fresh solution (benzene)
---- isomerized solution with iodine.

5) R. Kuhn and H. Roth, *Ber.*, **65**, 1285 (1932).
6) L. Zechmeister and W. A. Schroeder, *Arch. Biochem.*, **1**, 231 (1942).

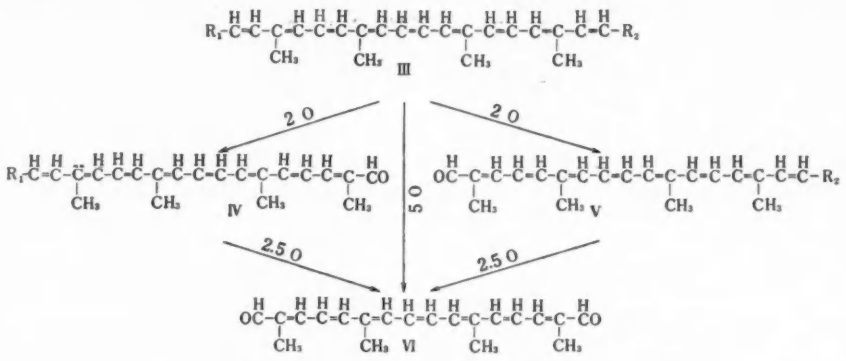


Fig. 6. Mild oxidation of renieratene.

fact indicates that they have 11 carbon-carbon double bonds in their molecules. Renieral exhibits the absorption maxima at 540 and 501 mμ in (carbon disulfide). Isorenieral exhibits absorption maxima at 526 and 489 mμ (in carbon disulfide) and gives phenylhydazone of m. p. 207°. Absorption curves in benzene with and without iodine are given by Fig. 3 and Fig. 4.

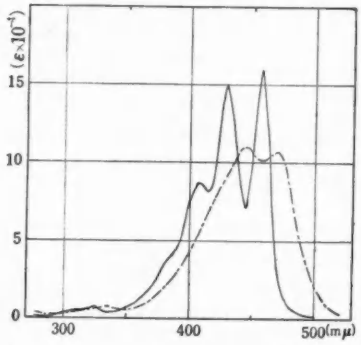


Fig. 5. Absorption curves of the pigment of m. p. 190°.
—— in petroleum ether,
---- in ethanol

When renieratene is oxidized with chromic acid corresponding to five atoms of oxygen, the principal product is the pigment of m. p. 190°, which forms orange red plates with purple reflex. The molecular formula of the pigment is $C_{20}H_{24}O_2$, and the absorption maxima are at 457, 430 and 407 mμ (in petroleum ether). The absorption curves are given in Fig. 5. As the pigment gives a bisphenylhydrazone of m. p. 239—242°, it seems to be a dialdehyde. These facts and the other properties of the pigment indicate that it is identical with crocetin dialdehyde, VI, (violet red plates, m. p. 190—191°, $C_{20}H_{24}O_2$, absorption maxima in petroleum ether, 458, 430 and 408 mμ; bisphenylhydrazone, m. p. 243°), which has been synthesized by Isler and coworkers[7]. It is for the first time that crocetin dialdehyde is obtained by the degradation of a carotenoid. Both renieral and isorenieral give crocetin dialdehyde by the oxydation with chromic acid corresponding to 2.5 atoms of oxygen. However, renieral does not give isorenieral on oxidation and neither does the reverse occur. These relationships are illustrated in Fig, 6, and the facts described above lead to the conclusion that renieratene, renieral and isorenieral are expressed by the partial formulae III, IV and V in Fig. 6, respectively. Both R_1- and R_2 groups seem to correspond to C_9H_{11}- which probably contain one ring and three double bonds.

From the positions of the absorption maxima of renieral and isorenieral, it is indicated that at least nine double bonds in these pigments should be in conjugation[8]. Therefore at least one double bond in R_1- and one double bond in R_2- group should be in conjugation with principal chromophore of renieratene. Numerous instances[9] of the mild oxidation of carotenoids have shown that the oxidative fission of double bond generally occurs at the outer end of the conjugated double bond system. However, in the case of renieratene, none of the pigments which have the absorption maxima at a longer

7) O. Isler, H. Gutmann, H. Lindlar, M. Montavon, R. Rüegg, G. Ryser and P. Zeller, *Helv. chim. Acta*, **39**, 463 (1956).
8) Calculated according to K. Hirayama's method, *J. Chem. Soc. Japan, Pure Chem. Sec.*, **75**, 674 (1954).
9) For example, R. Kuhn and C. Grundmann, *Ber.*, **65**, 900 (1932); R. Kuhn and H. Brockmann, ibid., **65**, 894 (1934); *Ann.*, **516**, 123 (1935).

wavelength region are obtained i. e. those which have a longer conjugated double bond system, than the above-mentioned aldehydes have. Therefore, in renieratene, the oxidation of double bond does not occur at the outer end of the conjugated system but at the inner position of the system. This indicates that the partial double bond systems contained in R_1- or R_2- group may be highly sablet towards oxidation, which also suggests the presence of benzene rings.

However, it seems to be contradictory to the view of the presence of benzene ring, that renieratene gives 7 moles of acetic acid on determination of side chain methyl groups. If renieratene had benzene rings in the molecule, non-volatile benzene polycarboxylic acid would be formed by such a serious oxidation as is employed in the determination; because the benzene ring in renieratene might be replaced by several methyl groups, as would be expected from the formula. In such a case, the amount of volatile acid should be no more than 4 moles when estimated as acetic acid. The account of this result can not be given in the present stage of the study.

Experimental

Renieratene prepared in the way described in the preceding papers[1,2] was employed. It formed fine purple red needles of m. p. 185° and was chromatographically homogeneous. Absorption maxima, 532, 496, 463 mμ (in carbon disulfide).

Anal. Found: C, 90.95; H, 9.26. Calcd. for $C_{40}H_{48}$: C, 90.85; H, 9.15%.

Infrared spectrum.—It was taken in a potassium bromide pellet, with a Perkin-Elmer 21 double beamins trument equipped with a potassium bromide prism (Fig. 2).

Molecular weight.—Signer's method[10] (method of isothermal distillation) was slightly modified. The solutions (128.1 mm³ and 194.1 mm³) of renieratene (5.913 mg. and 6.270 mg.) in benzene exhibited the same vapor pressures as those of the solutions (133.6 mm³ and 193.8 mm³) of benzil (2.531 mg. and 2.501 mg.) in benzene respectively. Found; 526.8, 536.7. Calcd. for $C_{40}H_{48}$, 528.8. In a parallel experiment on lycopene, 539.2 was obtained (Calcd. 536.9).

Catalytic hydrogenation (Kuhn and Möller's method[11]).—Pure sorbic acid was employed for the test substance. Catalyst, platinum oxide. Solvent, decalin-acetic acid (1:1). Found: 15.23, 15.17, 14.98 moles hydrogen. In parallel experiments, lycopene absorbed 13.03 moles hydrogen and β- carotene 10.94 moles hydrogen.

Isopropylidene group (Kuhn and Roth's method[5]).—On ozonolysis, renieratene (15.116 mg. and 8.912 mg.) gave acetone (0.077 mg. and 0.031 mg.) after subtraction of the blank value. These corresponded to 0.05 and 0.03 mole of acetone per mole of renieratene. In parallel experiments, lycopene and β-carotene gave 1.5 and 0.35 mole of acetone, respectively.

Side-chain methyl group (Kuhn and Roth's method[12]).—Renieratene (4.603 mg. and 4.196 mg.) gave volatile acid corresponding to 6.07 cc. and 5.73 cc. of 0.01 N sodium hydroxide solution. These corresponded to 6.97 and 7.22 moles of acetic acid. A parallel determination on lycopene gave 5.61 moles of acetic acid.

Vitamin A activity.—Renieratene was examined in experiments on four groups of rats in daily doses of 7, 15, 30 and 60 γ respectively. The rats of these four groups lost 26 g., 28 g., 27 g. and 26 g. (average on the rats of each group) in bodyweight, and finally died. The rats of the group for the positive control, which 7 γ of β-carotene were applied in daily doses, gained 33 g. on the average in body-weight in 30 days.

Cis-trans isomerization[13].—The solution (25 cc.) of renieratene (1.175 mg.) in benzene was prepared in a dark room, and 10 cc. of the solution was exposed to scattered light after the addition of iodine (ca. 6 γ) in benzene (0.8 cc.). The remainder was kept in the dark after the addition of benzene (1.2 cc.). After 30 minutes, 1 cc. of each solution was diluted to 25 cc. with benzene and the absorption curves were determined. They are given in Fig. 1. A remarkable *cis*-peak was ovserved at 362 mμ in the isomerized solution.

Chromic acid oxidation.—*a*) *Renieral and isorenieral.* Renieratene (133 mg.) was dissolved in a mixture of benzene (130 cc.) and acetic acid (130 cc.). To 10 cc. of this solution (containing ca. 5 mg. of the pigment), was added the mixture of the aqueous chromic acid (0.1 N, 0.2 cc.) and acetic acid (2 cc.) in one portion with vigorous stirring. The color changed into deep red. After 4—5 seconds the mixture was poured into water (20 cc.) covered with petroleum benzine (10 cc.), and washed with water several times. The procedure was repeated with other 10 cc. portions. All oxidized portions were combined together and dried with anhydrous sodium sulfate. The concentrated solution (30 cc.) was chromatographed on alumina (Merck) (column, 2.5 cm × 20 cm; developer, benzene) and gave the following zones in the sequence of decreasing adsorbability. 1) A thin yellow zone. It gave a small quantity of oily brown residue having absorption maxima at 454 and 425 mμ in petroleum benzine. 2) A thin yellow zone. It gave a small quantity of oily yellowish brown residue with absorption maxima at 455 and 425 mμ in petroleum benzine. 3) A thin orange zone. On treatment with petroleum benzine the residue afforded a minute quantity of red crystals having absorption maxima

10) R. Signer, ibid., **478**, 246 (1930).
11) R. Kuhn and E. E. Möller, *Angew. Chem.*, 145 (1934).
12) R. Kuhn and H. Roth, *Ber.*, **66**, 1274 (1933).
13) L. Zechmeister and A. Polgár, *J. Am. Chem. Soc.*, **65**, 1522 (1943).

at 456 and 429 mμ in petroleum benzine. It was crocetin dialdehyde. 4) A deep purple zone. After rechromatography on alumina, the residue of the eluate gave 8.5 mg. of renieral when recrystallized once from benzene-methanol. 5) A purplish red zone. After rechromatography on alumina and recrystallization of the residue of the eluate from petroleum benzine, 17.5 mg. of isorenieral was obtained. 6) A red zone. A small quantity of renieratene was recovered. Another experiment with 60 mg. of renieratene afforded 3.3 mg. of renieral and 7 mg. of isorenieral.

Renieral (further purified by recrystallization twice from benzene-methanol and twice from benzene-petroleum benzine) forms purplish brown needles, m. p. 198—199° (total 8.1 mg). It is soluble in benzene and difficultly soluble in petroleum benzine and methanol. Absorption maxima; 540, 501 mμ (in carbon disulfide); 499, 468 mμ (in hexane). Absorption curves (in benzene) with and without iodine (ca. 1.5% of the pigment) are given in Fig. III. On catalytic hydrogenation, 12.2 moles of hydrogen were absorbed.

Isorenieral (further purified by recrystallization thrice from petroleum benzine) forms brown crystals, m. p. 125—127° (12.5 mg.). It is soluble in benzene, petroleum benzine and hot ethanol, but is less soluble in methanol. Absorption maxima: 526, 489 mμ (in carbon disulfide); 486, 455 mμ (in hexane). Absorption curves (in benzene) with and without iodine are given in Fig. 4.

Anal. Found: C, 87.19; H, 8.88. Calcd. for $C_{30}H_{36}O$: C, 87.33; H, 8.80%.

On catalytic hydrogenation, 12.1 moles of hydrogen were absorbed. Phenylhydrazone, prepared by mixing the pigment (2 mg.) with phenylhydrazine in acetic acid and recrystallized once from benzene-methanol, formed purplish red needles, m. p. 207° (in an evacuated tube). It melted at 185° in an open capillary tube.

Anal. Found; N, 5.56. Calcd. for $C_{36}H_{42}N_2$; N, 5.57%.

b) Crocetin dialdehyde. Renieratene (120 mg.) was dissolved in a mixture of benzene (120 cc.) and acetic acid (120 cc.), and the solution was divided into 24 portions. Each portion (10 cc.) was oxidized with the mixture of the chromic acid solution (0.1 N, 0.5 cc.) and acetic acid (2 cc.), under vigorous stirring for one minute. The color of the solution changed into deep red and then gradually into orange yellow. The products were treated as in the case of a), and chromatographed on alumina. The main zone was orange red (it corresponded to the zone 3 of the chromatogram described in a)), while the zones of renieral and isorenieral were fairly faint. The residue of the eluate was recrystallized thrice from benzene-methanol and gave crocetin dialdehyde in purple red plates m. p. 190° (7.7 mg.). All the mother liquors of crystallizations of reneiral and isorenieral were combined together and oxidized as above with sufficient chromic acid to change the deep red color of the solution into orange yellow; 4.7 mg. of crocetin dialdehyde was further obtained. Absorption curve is given in Fig. 5.

Anal. Found: C, 81.46; H, 8.12%. Calcd. for $C_{20}H_{24}O_2$; C, 81.04; H, 8.16%.

Bisphenylhydrazone. Purple crystals, m. p. 256° (in an evacuated tube). In an open capillary tube, it melted at 239—242°.

Anal. Found; N, 11.79% Calcd. for $C_{32}H_{36}N_4$ 11.76%.

c) Oxidation of renieral. Renieral (0.8 mg.) was oxidized as above with chromic acid solution (0.1 N, 0.03 cc.), and the product was chromatographed. The principal zone gave a minute quantity of crocetin dialdehyde (m. p. 189°, mixed m. p. 189—190°).

d) Oxidation of isorenieral. Isorenieral (1.4 mg.) was oxidized with chromic acid solution (0.04 cc.). Crocetin dialdehyde was obtained as the main product (m. p. 189°, mixed m. p. 189—190°).

The author wishes to express his thanks to Professor Tokuichi Tsumaki for his exceedingly kind direction throughout this work. The author also thanks Mr. Kiyoshi Nakamura of Dainippon Pharmaceutical Co. Ltd. for the infra-red determination, Mr. Toshihiko Mukai and Mr. Yoichi Demura for their help in the collection of materials, and Mr. Michio Shido and Miss Sachiko Indo for microanalysis. The cost of this work has been partially covered by the Scientific Research Grant of Ministry of Education.

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SHORT COMMUNICATIONS

Decomposition of Aqueous Hydrogen Peroxide by Oxidized Chromia-Alumina Catalysts

By Yoshio MATSUNAGA

(Received August 7, 1957)

Our previous work[1] has established the fact that chromia-alumina catalysts contain an excess of surface oxygen after contact with air even at room temperature. In the extreme case, it has been shown that chromic ion can be oxidized completely to the plus-six state in sharp contrast with our knowledge of the thermal decomposition of chromic anhydride. Voltz and Weller[2] examined the catalytic decomposition of aqueous hydrogen peroxide by chromic oxide catalysts treated with oxygen at 500°C and suggested that the activities of the oxidized catalysts can be largely attributed to the chromic anhydride or its equivalent formed on the surface of these catalysts. However, their prediction was supported by measurements on three samples only, chromina gel, 20% chromia-alumina (Houdry Type R), and co-precipitated chromia-alumina. As we had many samples of various concentrations of chromium and also various degrees of oxidation, a more detailed examination of the relation between catalytic activity for the decomposition of aqueous hydrogen peroxide and the content of excess oxygen, has been undertaken.

The catalyst samples used in this study are those of the first series in our previous paper and the temperatures of oxygen treatments are 250°, 350° and 450°C. The content of excess oxygen, determined by an iodometric titration, ranges from 0.34 to 2.83 mg. eq./g. of catalyst and these amounts of excess oxygen cover the range of the mean oxidation number of chromium from 3.12 to 5.65. The volume of oxygen liberated by the reaction between 100 mg. of catalyst and 5.0 cc. of 30 % hydrogen peroxide (Edogawa Kagaku, Analytical Reagent) at 25°C was followed as a function of time for about ten minutes. The curves were found to be nearly straight. The catalytic activity represented by the volume of liberated oxygen per minute per g. of chromium is plotted against the concentration of chromium in Fig. 1. The curves bear a striking resemblance to those of the mean oxidation number as a function of chromium concentration shown in Fig. 1 of the previous paper. The above-mentioned activity is divided by the mean oxidation number of chromium and plotted against the concentration of chromium in Fig. 2.

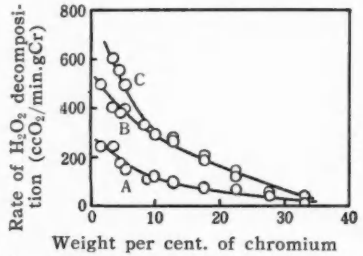

Fig. 1. Catalytic activity of oxidized chromia-alumina as a function of chromium concentration.
A; oxidized at 250°C, B; at 350°C, C; at 450°C.

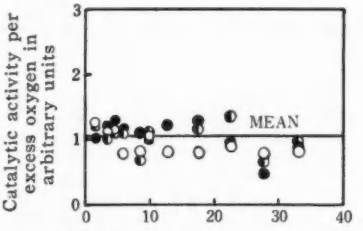

Fig. 2. Catalytic activity per excess oxygen in oxidized chromia-alumina as a function of chromium concentration.
○; oxidized at 250°C, ◑; at 350°C, ●; at 450°C.

It appears that the catalytic activity for a given amount of excess oxygen is nearly

1) Y. Matsunaga, This Bulletin, **30**, 868 (1957).
2) S. E. Voltz and S. W. Weller, *J. Am. Chem. Soc.*, **76**, 1586 (1954).

constant regardless of the mean oxidation number of chromium over the whole concentration range of chromium. In some of our catalyst samples the mean oxidation number of chromium exceeds plus five; therefore, we can conclude that the activity is largely due to the chromic anhydride formed on the surface of these catalysts. This conclusion is not only consistent with the suggestion given by Voltz and Weller, but also clarifies more definitely the nature of the surface of these catalysts.

The author wishes to express his hearty thanks to Professor H. Akamatu for his kind direction.

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Additions and Corrections

Volume 29, 1956

T. Tokuda: Atomic Weight of Oxygen in Quartz of Constrasted Geologic Origin.
Page 189. In col. 2, line 3 from the bottom, for "froat" read "float".
Page 190. In Table I, for "Locarity" read "Locality".
Page 191. In col. 1, line 4 from the bottom, for "Dork" read "Doak".

S. Yamada and T. Katō: Partition Equilibrium of Cumene Hydroperoxide between Water and Cumene.
Page 919. In col. 1, line 12 from the bottom, for "be" read "by".

Y. Matsunaga: Magnetochemical Study of Nitroso Compounds.
Page 971. In Table II, in col. 4, for "0.691" read "0.519".

Additions and Corrections

Volume 30, 1957

Y. Sasada and I. Nitta: A Refinement of the Crystal Structure of Tropolone Hydrochloride.
Page 63. In Fig. 1, for "(F_o-F_c)" read "$(\rho_o-\rho_c)$".
Page 64. In Fig. 2, add "$b/2$" at the upper left-hand corner.
Page 67. In col. 1, line 22 and 18 from the bottom, for "*m*-toluidine" read "*m*-tolidine".
Page 67. In Fig. 7(b), for "1.3" read "1.36".

M. Fujimoto: Mikroanalyse mit Hilfe von Ionenaustauschharzen. IX.
Page 83. In col. 2, line 25 from the bottom, for "Dowex 50 W" read "Dawex 50W".
Page 85. In Abb. 4, the mark ↑ should be placed on the right-hand ordinate.
Page 86. In col. 1, line 13, for "Fa ktoren" read "Faktoren".
Page 87. In col. 1, line 23, for "Stoffen" read "Stoffe".

M. Fujimoto: Mikroanalyse mit Hilfe von Ionenaustauschharzen. X.
Page 88. In col. 1, line 12 and 13 from the bottom, for "……harz" read "……harzes".
Page 88. In footnote 5), for "weise" read "Weise" and for "in den" read "in dem".

M. Fujimoto: Mikroanalyse mit Hilfe von Ionenaustauschharzen. XI.
Page 95. In Abb. 4, for "Harzkornchens" read "Harzkörnchens".

T. Hayakawa: The adsorption of Non-polar Gases on Alkali Halide Crystals. I.
Page 127. In col. 2, line 8 from the bottom, for "Fig." read "Figs.".

M. Iwasaki, S. Nagase and R. Kojima: The Molecular Structure of 1,2-Difluoro-1,1,2,2-tetrachloroethane.
Page 236. The author's address should be corrected as "Hirate-machi, Kita-ku,".

T. Hayakawa: The Adsorption of Non-polar Gases on Alkali Halide Crystals. II.
Page 241. In col. 1, line 12, for "Table" read "Tables"; in col. 2, line 26 from the bottom, for "Table" read "Tables".

T. Hayakawa: The adsorption of Non-polar Gases on Alkali Halide Crystals. III.
Page 244. In col. 1, the last line, for "Fig." read "Figs.".
Page 245. In col. 2, line 3, for "Fig." read "Figs.".

T. Hayakawa: The Adsorption of Non-polar Gases Alkali Halide Crystals. VI.
Page 346. In col. 1, line 4, for "covergence" read "convergence".

J. Higuchi: Primary Process Photochemical Decomposition of AH_N-Type Molecules.
Page 384. In col. 2, line 11 from the bottom, add "In the case of (2b), the equilibrium internuclear distance in the first excited state is not so great, but the crossing may take place near the equilibrium internuclear distance. Consequently, the actual potential energy curve to this decomposing direction may not have a maximum point and the first absorption spectrum occurs as continuous".
Page 388. In col. 2, line 25 from the bottom, for "$^1A_1\times A_1$" read "$^1A_1\times E$".

S. Iseda: On Mangiferin, the Coloring Matter of Mango (*Mangifera indica Linn.*). IV.
Page 626. In col. 1, line 3 from the bottom, omit "by" which precedes "driving".

S. Iseda: On Mangiferin, the Coloring Matter of Mango (*Mangifera indica Linn.*). IV.
Page 628. In col. 2, the last line, for "200°cc" read "200 cc".

S. Iseda: On Mangiferin, the Coloring Matter of Mango (*Mangifera indica Linn.*). V.
Page 631. In col. 2, line 20, for "euxantic" read "euxanthic".

S. Sakuraba: Studies on the Mechanism of Eder's Reaction. 1.
Page 666. In equation (5), for "H_2O_2" read "CO_2".

H. Naito and K. Sugawara: A Rapid Spectrophotometric Method for Determination of Minute Amount of Vanadium in Natural Waters.
Page 800. In col. 2, line 2 from end, word "he" should be followed after "and".
Page 800. The author's address should be corrected as "Chemical Institute, Faculty of Science Nagoya University, Chikusa-ku, Nagoya".